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**Vaccine Immunotherapy for Prostate Cancer**  
**David M. Lubaroff, PhD**  
**Principal Investigator**  
**Grant Number PC050647**  
**Final Report**

**INTRODUCTION:** The goal of this Clinical Trial Developmental Award (CTDA) was to develop and complete the required administrative tasks necessary to begin a Phase II clinical trial of an adenovirus/PSA (Ad/PSA) vaccine in men with recurrent prostate cancer and a Phase I clinical trial of the combination of the Ad/PSA vaccine along with immunostimulatory CpG ODN. The tasks included the design and construction of the clinical protocols, informed consent forms, submission to the institutional committees that include the Protocol Review and Monitoring Committee, Human Subjects Committee (IRB), Biosafety Committee, Pharmacy and Therapeutics Committee; the NIH Recombinant DNA Advisory Committee (RAC), and the Food and Drug Administration (FDA). In addition, although not funded by the CTDA, we were required by the FDA to perform Pharmacology/Toxicology and Histopathology Studies and to obtain a complete DNA sequence of the Ad/PSA vaccine.

**BODY:** We describe below the accomplishments during the one year award, using the original Statement of Work tasks.

Sequence the Ad5-PSA vaccine – The clinical grade Ad/PSA vaccine was submitted to Lark Technologies. The sequence report indicated that there were no additions to the normal sequences of the PSA insert nor to the adenovirus backbone.

Develop protocols, investigator brochures, and IRB forms for the Phase II trial of the Ad/PSA vaccine alone – This task consumed the great majority of time during the one year of the award. Multiple meetings were held among the Principal Investigator (PI) and members of the Clinical Trial Team to make important decisions about the trial protocols. The discussion point included the patient populations to be targeted in the trials, the eligibility and exclusion criteria, the primary and secondary endpoints, and times for follow-up visits. The plans for the trial were presented to large groups of basic scientists and clinicians, the results of which enhanced the final protocols. In addition, a number of meetings were held during the year with two biostatisticians in the Biostatistics Core of the Holden Comprehensive Cancer Center. In the end, two protocols, targeting three separate patient populations, were completed for the Phase II trial. Copies of the Phase II protocols are attached. The Investigator's Brochure was also constructed and it too, is attached to this report. Submissions for institutional approval were completed and are currently under review by the Protocol Review and Monitoring Committee. Similarly, we have submitted our application to the University of Iowa's IRB and they are currently reviewing the submission. Additional committees have also received our applications that include the Pharmacy and Therapeutics and Biosafety Committees. An application to the NIH RAC was submitted and we received a notice that we are exempted from full committee and public review of the protocols. Copies of those notices are attached.

Develop protocols, investigator brochures, and IRB forms for the Phase I trial of the Ad/PSA plus CpG ODN – This process has been delayed due to the ongoing negotiations between our clinical trial team, Coley Pharmaceuticals Group, and Pfizer, Inc. Coley was the company that initiated clinical trials of the immunostimulatory CpG ODN, but has since out-sourced all of their cancer vaccine work to Pfizer. Thus, until an agreement can be reached, we have delayed our Phase I trial of the Ad/PSA plus CpG ODN.

Analyze data from FDA-required required studies (not funded by the Clinical Trial Development Grant) – We are required to perform pharmacology/toxicology and histopathology studies in mice injected with the three injection prime-boost strategy we propose for the Phase II trial. Mice were injected with  $10^8$  pfu of the Ad/PSA vaccine in a collagen matrix, a dose determined by the results of our completed Phase I trial. At each of the 8 follow-up time periods after vaccine injections for the study – days 0, 30, 33, 44, 60, 63, 74, and 90, we examined the injection site for evidence of local toxicity, bled the mice, and removed selected organs for study as described below. We also examined the mice daily for overt signs of clinical toxicity such as ruffled fur, posture, and activity. Each mouse was weighed twice per week. Blood was collected and used to measure AST, ALT, LDH, alkaline phosphatase, bilirubin, glucose, total protein, BUN, and creatinine. Tissues that were analyzed for histopathology: injection site, draining lymph node, contralateral lymph node, liver, lung, spleen, heart, brain, gonads, prostate, urinary bladder, kidney. The data obtained from these studies demonstrated that no abnormalities were found in any of the 11 tissues examined and normal values were evident throughout the study period in the analyses for blood cells, renal function, and liver function.

Arrange pre-IND conference for the Phase I trial – Postponed as the result of delayed negotiations with Pfizer. See explanation above.

Prepare and submit IND application for Phase I trial - Postponed as the result of delayed negotiations with Pfizer. See explanation above.

Receive approval from the FDA for Phase I trial - Postponed as the result of delayed negotiations with Pfizer. See explanation above.

Prepare and submit amendment to current IND for Phase II trial – This is in progress. We have requested a “no cost” extension to the CTDA to accomplish this task as well as the following task. As soon as we receive approvals from our institutional committees we will submit the FDA amendment.

Receive approval from the FDA for Phase II trial – Pending completion of the submission task above.

Finally, although not a task not outlined in the original CTDA application, we have submitted an application to the Department of Defense’s Prostate Cancer Research Program for a Clinical Trial Award to fund the Phase II trial.

#### KEY RESEARCH ACCOMPLISHMENTS:

- Completed two clinical protocols for the Phase II trial of the Ad/PSA vaccine
- Submitted protocols to University of Iowa institutional committees – Protocol Review & Monitoring, IRB, Biosafety, and Pharmacy & Therapeutics.
- Prepared and submitted application to NIH RAC.
- Received exemption from full RAC review of the protocols
- Sequenced the DNA of the vaccine.
- Performed pre-clinical pharmacology/toxicology and histopathology studies.
- Began negotiations with Pfizer, Inc. for joint Phase I clinical trial of the Ad/PSA vaccine plus CpG ODN.

REPORTABLE OUTCOMES INCLUDED IN APPENDICES: Attached are two clinical protocols, the investigator’s brochure, notice of RAC exemptions, and the first two pages of the Clinical Trial Award

application submitted June 20, 2006. The completed grant application can be obtained from the Department of Defense.

**CONCLUSION:** We have successfully prepared our submission to regulatory agencies following a year of discussions and biostatistical consultation. We have received an exemption from the RAC and are awaiting institutional approval before submitting our amendment to the FDA.

**PHASE II STUDY OF ADENOVIRUS/PSA VACCINE IN MEN WITH RECURRENT  
PROSTATE CANCER AFTER LOCAL THERAPY**

Version 12 – June 8, 2006

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## 1. INTRODUCTION

### 1.1 Background- Immunotherapy in Prostate Cancer

Prostate cancer is the second leading cause of cancer death among males in the United States. There will be an estimated 234,460 new diagnoses of prostate cancer made in the United States in 2006 (1) (the estimates for 2006 were not available at the time of the protocol completion). Treatments for organ-confined prostate cancer include radical prostatectomy and radiation therapy. When the cancer presents de novo, or recurs outside the prostate, first-line systemic treatments typically include hormonal blockade (with LHRH agonists or bilateral orchiectomy), which suppress testosterone levels, limit the growth of androgen-dependent cancer cells, and result in clinical tumor control. After a median time of 2 years, patients progress into a clinical hormone-refractory state, when the prostate specific antigen (PSA) levels rise despite castration, there is proliferation of androgen-independent cancer cells, and there is continued clinical tumor growth that becomes fatal. Therapeutic measures in this situation include further hormonal manipulations or the use of systemic chemotherapy, which has recently shown a small survival benefit in phase III trials. Approximately 30,000 Americans die from prostate cancer each year.

Immunotherapeutic approaches against prostate cancer have been investigated for several years. Most of these studies have concentrated on active non-specific therapy and adoptive or passive therapy, with only recent focus on the induction of antigen-specific immune responses. Viral vectors have been used successfully in both gene transfer and vaccine therapy studies (2). Replication-competent and replication-deficient adenoviruses expressing foreign proteins have been used to elicit immune responses to a variety of tumor antigens (3-7).

We have demonstrated that immunizations with adenovirus, carrying the human PSA gene, can induce vigorous anti-PSA T-cell responses and cause the destruction of PSA-secreting tumors in a pre-clinical mouse model of prostate cancer (8,9). Such active immunization against prostate-cancer associated antigens might be more effective than active non-specific or adoptive/passive immunotherapy. Therefore, we have pursued a vaccination strategy based on an adenovirus that carries the gene for prostate specific antigen (PSA). Results from our Phase I trial of adenovirus/PSA (Ad/PSA) vaccine (section 1.4, below) demonstrated that a single immunization of men with metastatic prostate cancer was able to induce anti-PSA T cell responses. The trial design was a dose escalation study with the vaccine administered subcutaneously (sc) either in an aqueous solution or in a collagen matrix (Gelfoam®). We now propose a Phase II clinical trial using the Ad/PSA vaccine, administered in multiple injections to prostate cancer patients with minimal disease burden.

### 1.2 Adenovirus vectors

Recombinant adenoviral vectors transduce a wide range of dividing and nondividing cells types, making this gene delivery system valuable as a tool for studying diseases, for vaccine therapy, and for potential clinical use (10). Recombinant adenovirus can be prepared and purified in high titers. In addition, wild-type adenovirus infections are extremely common in the general population, giving adenovirus a well-documented safety record (11). Moreover, adenoviruses are structurally stable and no adverse effects have been reported following the vaccination of US military recruits with wild types, demonstrating their safety for human use (11). Adenoviral vectors for gene therapy and vaccine therapy are adenoviruses which have been genetically modified to allow insertion of foreign genes and to render the virus replication-defective. Current vectors have a deletion in the E1 region or in both the E1 and E2 regions.

Adenoviral gene transfer has been used in a variety of experimental conditions that include transfers to the liver (12), lung (13), central nervous system (14,15), and to cancer cells (16).

There is evidence that the introduction of foreign transgenes by adenovirus induces immune responses to the transgene product, which become ultimately responsible for the elimination of the virus (17,18). While this is disadvantageous for insertion of functional genes into host cells, it is advantageous in the use of viruses carrying foreign genes as immunogens. In the vaccine therapy of cancer, active immunization against a murine colon cancer, breast cancer, and melanoma antigens have been induced by adenoviral vaccines (19-25).

The Ad/PSA vaccine used our laboratory and in our Phase I clinical trial was produced by inserting the gene for the full length pre-pro form of human PSA into a replication deficient adenovirus serotype 5. Replication deficiency was induced by deletion of the E1a and E1b genes of the virus. Details of the vaccine can be found in section 9 of this protocol. Approval for the use of the vaccine in the Phase I trial was obtained from the FDA under IND #9706.

In pre-clinical studies, our group has demonstrated that the Ad/PSA vaccine was able to induce stronger anti-PSA immune responses than other viral PSA vaccines. These include vaccinia viruses, both replication competent and replication deficient, and to a canarypox vaccine (Table 1). The frequency of PSA-specific CD8+ cells T cells generated by the Ad/PSA vaccine was greater than were generated by any of the other vaccines tested.. In addition to the superior immunizing property of the Ad/PSA, the incorporation of Gelfoam, a collagen matrix (section 1.3), has been shown in pre-clinical studies to enhance the ability of the vaccine to induce strong anti-PSA immune responses (8). Lastly, immunization of mice with Ad/PSA in matrix can induce anti-PSA responses even in the presence of high titer anti-adenovirus antibodies (8). This latter finding is important in light of the fact that most humans have pre-existing levels of anti-adenovirus antibodies as a result of prior natural exposure to the virus.

**Table 1**  
**Effector Cell Frequency Analysis (ELISPOT)**

<b>Vaccine</b>	<b>Virus</b>	<b>Frequency of PSA-Specific CD8+ T Cells</b>
Ad/PSA*	Replication deficient adenovirus	1/455
Prostvac	Replication competent vaccinia	1/2028
NYVAC/PSA	Replication deficient vaccinia	1/3597
ALVAC/PSA	Canarypox	1/35,714

### 1.3 Gelfoam® Matrix

Gelfoam (Pharmacia & Upjohn Company, Kalamazoo, MI) is a medical device intended for application to bleeding surfaces as a hemostatic agent. It is a water-insoluble, off-white, non-elastic, porous, pliable product prepared from purified pork skin. The Gelfoam gelatin preparation is available either as a cross-linked sponge or as non-cross linked beads. It is able to absorb and hold within its interstices approximately 45 times its weight of blood and other fluids (26). The absorptive capacity of Gelfoam is a function of its physical size, increasing with increasing gelatin volume (27).

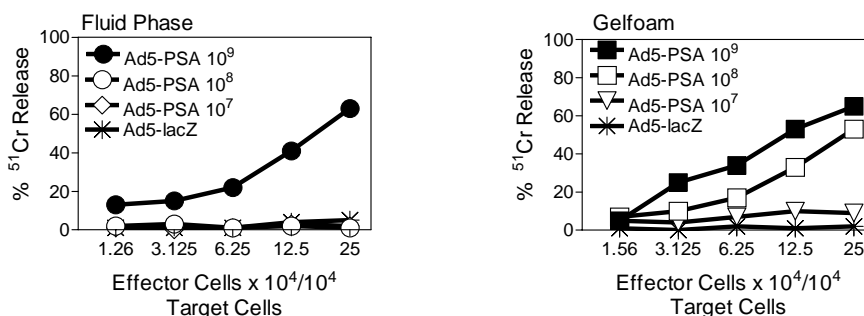
The mechanism of action of surface-mediated hemostatic devices is supportive and mechanical (27). Surface-acting devices, when applied directly to bleeding surfaces, arrest bleeding by the formation of an artificial clot and by producing a mechanical matrix that facilitates clotting (28). Jenkins et al have theorized that the clotting effect of Gelfoam may be due to release of thromboplastin from platelets, occurring when platelets entering the Gelfoam become damaged by contact with its myriad of interstices (29). Thromboplastin interacts with prothrombin and calcium to produce thrombin, and this sequence of events initiates the clotting reaction. The authors suggest that the physiologic formation of thrombin in Gelfoam is sufficient to produce formation of a clot, by its action on the fibrinogen in blood (29). The spongy physical properties of Gelfoam hasten clot formation and provide structural support for the forming clot (28,30).

Gelfoam has been used experimentally for the delivery of soluble proteins and drugs, including insulin, antibiotics, and growth factors (31-33). Gelfoam was used for sustained release of insulin in an ocular implant device (31). Delivery of insulin in solution had no effect on blood glucose levels. In contrast, the use of Gelfoam as a sustained release delivery agent provided measurable insulin activity for up to 10 hours after implantation. Glucose levels in the blood stabilized at 60% of the original value, whereas administration of insulin in eye drops had no effect.

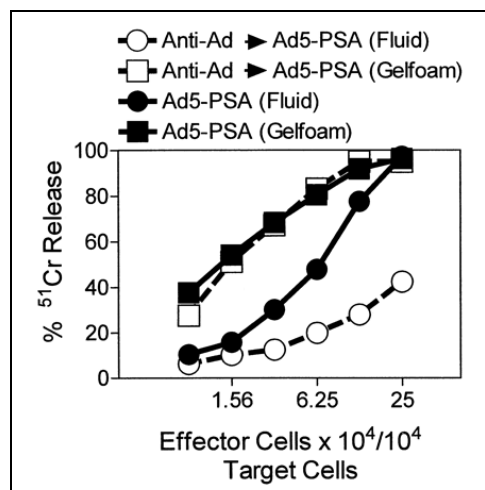
MacDonald and Mathews (34) studied Gelfoam implants in canine kidneys and reported that it assisted in healing, with no marked inflammatory or foreign-body reactions. Jenkins and Janda (35) studied the use of Gelfoam in canine liver resections and noted that Gelfoam appeared to offer a protective cover and provide structural support for the reparative process. Correll et al (36) studied the histology of Gelfoam when implanted in rat muscle and reported no significant tissue reaction.

Gelfoam has been used as a hemostatic agent in dog prostate (37). In these studies no gross histological evidence of tissue damage or calcification was induced. In addition, these investigators demonstrated that placement of Gelfoam into the lumen of the bladder resulted in liquefaction of the Gelfoam without any evidence of calculogenesis. Finally, Bischoff and Goerttler (38) used Gelfoam in human prostate therapeutic embolization with success.

Our laboratory, in collaboration with Dr. Timothy Ratliff, has demonstrated that administration of the Ad/PSA vaccine in Gelfoam induces a stronger anti-PSA immune response (Figure 1). In our pre-clinical studies, immunization with the vaccine in an aqueous suspension induces strong immunity with  $10^9$  pfu with weaker immunity induced with  $10^8$  and  $10^7$  pfu. Use of Gelfoam permits the induction of strong responses at the lower dose of  $10^8$  pfu. In addition, strong anti-PSA T cell responses could be induced by immunization with the Ad/PSA vaccine in Gelfoam even in mice pre-immunized to adenovirus (Figure 2). In the Phase I clinical trial (section 1.4), the addition of Gelfoam to the vaccine immunization did not result in excess serious adverse events.



**Figure 1** – Anti-PSA cytotoxic activity of spleen cells obtained from mice immunized with Ad/PSA in aqueous (fluid phase) versus Gelfoam.



**Figure 2.** Effect of the presence of anti-adenovirus Ab on Ad5-PSA immunization with and without Gelfoam. Mice were injected i.p. with  $10^9$  PFU of Ad5-lacZ or PBS 2 wk before immunization with  $10^9$  PFU Ad5-PSA delivered s.c. in the fluid phase (PBS) or Gelfoam. CTL were tested against RM-11psa and RM-11neo to demonstrate PSA specificity. Cytolytic activity of each group against RM-11neo targets was <10% for all E:T ratios.

#### 1.4 Phase I study

A Phase I clinical trial of the Ad/PSA vaccine has been completed in men with measurable metastatic prostate cancer, with the primary objectives of determining the toxicity profile and maximal tolerated dose (MTD). The ability of the vaccine to induce anti-PSA immune responses and any clinical responses was also evaluated.

Eligible patients consisted of men with prostate cancer that had measurable metastatic disease, 90% of whom were stage D3. Prior therapies had included androgen withdrawal, ketaconazole, prednisone, Casodex, Taxotere, and external beam radiation, but the initiation of vaccine therapy was equal to, or greater than, 30 days after the most recent therapy. Patients were treated in successive dose levels and aqueous vs. matrix cohorts, according to the protocol plan. We were able to administer the maximum permitted dose of  $10^8$  pfu without any serious adverse events by treatment of the first of 18 patients. These initial 18 patients were followed throughout the one-year period after injection. An additional 14 patients were treated at the MTD dose level, as planned in the protocol and confirmed by a letter to the FDA. The purpose of the additional patients was to have sufficient numbers of patients in the groups to statistically evaluate the anti-PSA immune responses induced by the Ad/PSA vaccine. In summary, 32 patients were treated in the study followed through the one-year period. Two additional patients were enrolled in the study (total number of enrolled patients, 34) but never received the vaccine and were therefore not evaluable. One patient chose to have radiation therapy instead of participating in the trial and the second was diagnosed with a second malignancy (melanoma) shortly after his enrollment in the phase I study.

##### 1.4.1 Phase I study results

The median age of the patients was 70.2 years (range, 52 to 89). The vaccine was administered as an aqueous suspension or in a collagen (Gelfoam) matrix to 32 patients. Sixteen (16/32) or 50% of the patients exhibited grade 1 vaccine-related adverse events (AE), 1/32 (3.1%) that exhibited a grade 2 AE, and one patient exhibited a grade 3 AE which was a decrease in neutrophil count. There were no vaccine-related grades 4 or 5 AEs.

We measured the anti-PSA immune responses, both antibody and T cell, in all patients enrolled in the study. Antibody responses to PSA were measured by the binding to PSA-

secreting cell lines using the method adapted from Cavacini, et al. (39). Results of those analyses demonstrated that 57% of men immunized with the Ad/PSA vaccine developed measurable anti-PSA antibodies. ELISPOT assays were utilized to measure anti-PSA T cell responses. The results, depicted in Table 2, demonstrate that of the 32 patients, 18 (56.3%) developed anti-PSA T cell responses. The addition of Gelfoam did not appear to affect the development of anti-PSA responses, but in this Phase I study the numbers of patients in each group was too small to make statements of statistical significance of the data. These results demonstrate the ability of men with late stage metastatic prostate cancer, injected one time with Ad/PSA, to respond to the vaccine with the production of anti-PSA T cells.

**Table 2**  
**ELISPOT Analysis of Anti-PSA T Cell Immune Responses**

Patient Number	Dose/ Vehicle	Response	Frequency	
			Pre-Immunization	Post-Immunization
AP-002	10 <sup>6</sup> -aqueous	-	1/985,000	1/258,571
AP-004	10 <sup>6</sup> -aqueous	-	0	1/311,111
AP-007	10 <sup>6</sup> -aqueous	+	1/46,901	1/14,804
AP-003	10 <sup>6</sup> -matrix	+	1/90,000	1/8075
AP-005	10 <sup>6</sup> -matrix	+	1/1.2x10 <sup>6</sup>	1/152,353
AP-006	10 <sup>6</sup> -matrix	+	1/93,103	1/15,762
AP-008	10 <sup>7</sup> -aqueous	-	1/100,000	1/97,419
AP-010	10 <sup>7</sup> -aqueous	-	0	0
AP-013	10 <sup>7</sup> -aqueous	+	0	1/60,000
AP-009	10 <sup>7</sup> -matrix	+	1/52,535	1/30,566
AP-012	10 <sup>7</sup> -matrix	-	1/13,488	1/254,286
AP-014	10 <sup>7</sup> -matrix	+	1/562,500	1/120,000
AP-015	10 <sup>8</sup> -aqueous	+	1/1.4x10 <sup>6</sup>	1/780
AP-016	10 <sup>8</sup> -aqueous	+	0	1/130,000
AP-017	10 <sup>8</sup> -aqueous	+	0	1/124,375
AP-025	10 <sup>8</sup> -aqueous	-	0	0
AP-026	10 <sup>8</sup> -aqueous	-	0	1/560,000
AP-027	10 <sup>8</sup> -aqueous	+	0	1/26,364
AP-029	10 <sup>8</sup> -aqueous	+	0	1/333
AP-032	10 <sup>8</sup> -aqueous	+	1/250,000	1/3793
AP-018	10 <sup>8</sup> -matrix	-	0	0
AP-019	10 <sup>8</sup> -matrix	+	1/14,235	1/336
AP-020	10 <sup>8</sup> -matrix	+	1/8824	1/1802
AP-021	10 <sup>8</sup> -matrix	+	1/689	1/431
AP-022	10 <sup>8</sup> -matrix	+	0	1/180,000
AP-023	10 <sup>8</sup> -matrix	-	1/320,000	0
AP-030	10 <sup>8</sup> -matrix	+	1/666.667	1/32,000
AP-034	10 <sup>8</sup> -matrix	+	1/80,000	1/15,152
AP-035	10 <sup>8</sup> -matrix	+	1/5295	1/2459
AP-036	10 <sup>8</sup> -matrix	+	1/62,000	1/9487
AP-037	10 <sup>8</sup> matrix	-	1/2.1x10 <sup>6</sup>	1/965,000

The effects of vaccination on serum PSA and on patient survival were also evaluated as a secondary endpoint in the phase I trial. Although there was no sustained decline in individual PSA levels, the PSA doubling times (PSADT, calculated based on 3 pre-enrollment consecutive PSA measurements) were reduced in 54 percent of the patients compared to pre-vaccine administration, with the best responses occurring in patients immunized with the highest dose of

the vaccine (Table 3). In addition, published survival nomograms for patients with hormone refractory prostate cancer were applied to patients in this phase I trial (40,41). Table 4 shows that 57% of all patients at all doses, whether injected with the vaccine as an aqueous suspension or in the collagen matrix, had a survival time longer than that predicted by the nomogram. The range of increased survivals in the different groups was 33% to 100%.

**Table 3**  
**PSA Doubling Time (DT) in Phase I Patients**

<b>Vaccine Dose &amp; Vehicle</b>	<b>Percent Increased PSA DT</b>
10 <sup>6</sup> aqueous	33%
10 <sup>6</sup> matrix	33%
10 <sup>7</sup> aqueous	67%
10 <sup>7</sup> matrix	67%
10 <sup>8</sup> aqueous	25%
10 <sup>8</sup> matrix	62%
<b>Total</b>	<b>54%</b>

**Table 4**  
**Ad/PSA Phase I Trial**  
**Predicted and Actual Patient Survival**

<b>Vaccine Dose</b>	<b>Vehicle</b>	<b>Percent with Longer Than Predicted Survival</b>
10 <sup>6</sup>	Aqueous	33%
10 <sup>6</sup>	Matrix	67%
10 <sup>7</sup>	Aqueous	100%
10 <sup>7</sup>	Matrix	33%
10 <sup>8</sup>	Aqueous	56%
10 <sup>8</sup>	Matrix	50%
<b>Overall</b>		<b>57%</b>

### 1.5 Other immunotherapy clinical trials for prostate cancer

The last several years have seen an increase in the number of clinical trials using vaccine immunotherapy for the treatment of prostate cancer. The trials have used a variety of target antigens that have been shown to be associated with prostate and prostate cancer cells. These include PSA (39,42-49), prostatic acid phosphatase (PAP) (50-53), prostate specific membrane antigen (PSMA) (54-56), telomerase (hTERT) (57,58), Thomsen-Friedenreich antigens (59), mucins (60), carbohydrates (61), and HLA-associated peptides (62). A variety of vectors have been used in the immunization process that include dendritic cells (45,50-58,63), vaccinia virus (39,42,43,47,49), fowlpox virus (39,47), liposomes (44), plasmids, (48), and chemical conjugates (59-61).

Recently, Kaufman et al. recently reported the results of a phase II trial (ECOG 7897) with a prime/boost vaccine using vaccinia virus and fowlpox virus expressing human PSA in patients with hormone-dependent prostate cancer (64). Sixty-four eligible patients with biochemical progression after local therapy were randomly assigned to three treatment arms: (A) fowlpox-PSA (rF-PSA) by intramuscular injection every six weeks for four doses, (B) rF-PSA for three doses followed by vaccinia-PSA (rV-PSA) given by intradermal injection, or (C) rV-PSA followed

by three rF-PSA vaccines. Dreicer et al. reported a randomized phase II study with a recombinant Modified Vaccinia Ankara virus which expresses both MUC1 and IL2 (TG4010) (65). MUC1 is a glycoprotein associated with several malignancies. Eligible patients were required to have no evidence of metastatic disease following curative intent local therapy, evidence of PSA failure (PSA over 2 ng/ml) and PSA doubling time (PSA-DT) less than 10 months. Arm 1 had TG4010 injected sc weekly, at a dose of  $10^8$  pfu, for six weeks, then every three weeks. Arm 2 had  $10^8$  pfu TG4010 injected sc every three weeks. Therapy was continued to disease progression or to a maximum of 36 weeks. Lastly, Small et al. recently presented the results of a phase III trial with APC8015, an immunotherapy cellular product consisting of autologous peripheral blood mononuclear cells enriched for a dendritic cell fraction pulsed with PA2024, a Prostatic Acid Phosphatase (PAP)-GM-CSF construct (66). Patients with asymptomatic, metastatic hormone-refractory prostate cancer were randomized (2:1) to receive APC8015 (n=82) or placebo (n=45) every 2 weeks x 3.

ECOG is currently planning a phase III trial using the Vaccinia virus (PROSTVAC-V/TRICOM) followed by Folwipox virus vaccination (PROSTVAC-F/TRICOM) with GM-CSF, compared with placebo vaccine plus GM-CSF in patients with hormone-refractory prostate cancer with absence of metastatic disease (ECOG 1805, PARADIGM).

In summary, the results from these trials vary in terms of patient populations studied (hormone dependent vs. independent) and in levels of positive results, which include the induction of antigen-specific immune responses, decreases in levels of serum PSA and in rates of change in PSA velocity, and measures of clinical responses. Thus far no single vaccine immunotherapy has proven to be definitely superior to others in terms of clinical benefit, and other phase II and III trials continue to be planned or conducted. The results of some of these vaccine trials raise the question that an increase in PSADT may in the future represent a possible surrogate marker for increased time to progression, or overall survival in immunotherapy studies, and that absolute PSA responses may not constitute an obligatory step for the ultimate demonstration of clinical benefit of immunotherapy approaches in prostate cancer. Furthermore, the T-cell stimulation index may have important correlation with clinical vaccine efficacy, as seen in the phase III trial by Small et al.(66). These developing notions further support the current proposal for clinical development of our Ad/PSA vaccine, also based on the results of our prior phase I trial.

#### 1.6 Proposed phase II clinical trial: rationale

Based on the significant pre-clinical activity of the Ad/PSA vaccine in generating tumor-specific T cells, and the encouraging safety and efficacy results from our phase I study, we propose to continue the clinical development of the Ad/PSA vaccine with the performance of the current phase II trial. It is our contention that the vaccine product and the method of immunization set this therapy apart from other ongoing prostate cancer investigational immunotherapeutic approaches. Specifically, the incorporation of Gelfoam, not present in other vaccine preparations, enhances the induction of strong anti-PSA responses. Immunization of mice with Ad/PSA in Gelfoam matrix was able to induce anti-PSA responses even in the presence of high-titer anti-adenovirus antibodies. Notably, most humans naturally possess high titers of anti-adenovirus antibodies due to natural exposure to adenoviruses.

We plan to enroll prostate cancer patients into one of two arms (A & B) of the Phase II clinical trial. The ideal patient population to determine a therapeutic benefit of a new treatment, particularly immunotherapy, is one with minimal disease burden. The low tumor burden should allow therapies, particularly those relying on antigen-specific effector T lymphocytes, to destroy

all of the cancerous tissues and cells. The first therapeutic arm (Arm A) will enroll men with recent evidence of recurrence following surgery or radiation therapy for their primary tumor. Patients in the population will be eligible if they exhibit at least four separate rises in serum PSA, at least one month apart with differences  $\geq 0.03$  ng/ml and a total PSA of  $>0.2$  ng/ml; have a PSA doubling time of  $\geq 6$  months; not at high risk or patients that refuse treatment with Taxotere or radiation. A high risk patient will be defined as those with a serum PSA of  $>20$  ng/ml and a Gleason score of  $>7$ . All patients will be hormone naïve. Since standard therapy for these patients would be to postpone androgen ablation therapy until such time as there is a high serum PSA level ( $\geq 20$  ng/ml), enrolling patients into this Phase II trial does not withhold accepted treatment. Patients will be excluded from the trial if they are candidates for salvage radiation therapy, had multiple positive margins at surgery, had a serum PSA of  $>20$  ng/ml prior to surgery, a Gleason score of  $>7$ , seminal vesicle involvement or positive lymph nodes.

The second therapeutic arm (Arm B) will enroll men with recurrent disease who are undergoing androgen depletion therapy. The choice of this additional patient population is based upon published documentation that inflammation and the generation of immune responses are augmented by hormone withdrawal (67-69). Mercader, et al., in attempts to demonstrate an enhanced termination of tolerance to prostate associated antigens documented CD4+ and CD8+ T cell infiltrates in benign prostates and in prostate tumors of men undergoing androgen withdrawal (67). Roden and co-workers published data demonstrating that T cell levels and T cell proliferation were increased in mice following castration (68) while Drake, et al. reported breaking tolerance to antigens associated with the TRAMP prostate tumors in mice (69). Therefore, we propose to vaccinate men beginning 14 days after the initiation of androgen depletion therapy using the same three injection protocol.

## 2. OBJECTIVES

### 2.1 Primary Objective

To evaluate the development of anti-PSA immune responses in study patients, of particular importance is the comparison of immune responses generated in the hormone naïve and AWT patients.

### 2.2 Secondary objectives

- 2.2.1 To evaluate the response rates (PSA responses and changes in PSADT) of the Ad/PSA vaccine using a prime-boost immunization strategy, in patients with recurrent disease, either hormone naïve or during androgen deprivation therapy (ADT).
- 2.2.2 To evaluate biochemical (PSA recurrence) and radiographic (bone scans) time to progression and overall survival in evaluable patients receiving the Ad/PSA vaccine.

## 3. SELECTION OF PATIENTS

As described in Section 1.6 prostate cancer patients will be enrolled in one of two arms of the study; men with recurrent disease who are hormone naïve (Arm A) and patients who have begun hormone therapy (Arm B), vaccination in the latter group to be initiated fourteen days after the start of therapy.



### 3.1 Inclusion criteria:

3.1.1 Men with prostate cancer who have received prior local therapy (radical prostatectomy or definitive radiation therapy) and have biochemical (PSA) relapse without evidence of radiographic or clinical metastatic disease.

3.1.2 For men who had prior prostatectomy, the surgery must have occurred at least 6 months prior to study enrollment.

3.1.3 For men who had prior definitive radiation therapy, radiation must have occurred at least 1 year prior to study enrollment.

3.1.4 Exhibit at least four separate rises in serum PSA, at least one month apart with differences  $\geq 0.03$  ng/ml and a total PSA of  $>0.2$  ng/ml.

3.1.5 Have a PSA doubling time of  $\geq 6$  months.

3.1.6 Not at high risk as defined as those with a serum PSA of  $>20$  ng/ml and a Gleason score of  $>7$ . or patients that refuse treatment with Taxotere or radiation

3.1.7 Negative bone scans.

3.1.8 Negative CT scans of chest abdomen and pelvis (no soft tissue metastases present).

3.1.9 Scans must be obtained within 6 weeks of entry into the trial.

3.1.10 Written informed consent.

3.1.11 Age  $\geq 18$  years.

3.1.12 Required laboratory values (obtained within 2 weeks of study entry)

3.1.12.1 Serum creatinine  $\leq 2.0$  mg/dL

3.1.12.2 Adequate hematologic functions: granulocytes  $\geq 1800$  per  $\text{mm}^3$  and platelets  $\geq 100,000$  per  $\text{mm}^3$

3.1.12.3 Adequate hepatocellular function: AST  $<3$ x normal and bilirubin  $<1.5$  mg/dl.

### 3.2 Exclusion criteria:

3.2.1 Candidates for salvage radiation therapy.

3.2.2 Had multiple positive margins at surgery.

3.2.3 Had a serum PSA of  $>20$  ng/ml prior to surgery.

3.2.4 Gleason score of  $>7$ .

3.2.5 Seminal vesicle involvement or positive lymph nodes.

3.2.6 Active or unresolved infection.

3.2.7 Parenteral antibiotics <7 days prior to study entry.

3.2.8 Evidence of prior or current CNS metastases. Specific imaging is not necessary in the absence of signs or symptoms.

3.2.9 Co-morbid medical conditions which would result in a life expectancy (participation) of less than 1 year.

3.2.10 Patients with compromised immune systems; congenital, acquired, or drug-induced (immunosuppressive agents) will be excluded from the study. Use of prednisone at doses higher than 10 mg daily (or equipotent steroid doses) for more than 7 days within the last 3 months is not allowed.

3.2.11 No-pre-existing malignancies that required treatment within the past 5 years except for basal or squamous cell cancers of the skin.

3.2.12 Prior systemic therapies for prostate cancer not allowed (hormonal therapy, including but not limited to LHRH agonists, antiandrogens, ketoconazole or chemotherapy); only patients in Arm B, undergoing androgen depletion therapy during the vaccination will be eligible.

3.2.13 Prior participation in any vaccine studies for any disease.

#### **4. Registration Procedures**

4.1 All patients will be registered through the Department of Urology at the University of Iowa Hospitals and Clinics.

4.2 Patients who are candidates for enrollment into the study will be evaluated for eligibility by the clinical investigators to ensure that the criteria outlined in Section 3 have been satisfied and that the patient is eligible for participation in this clinical investigation. The University of Iowa will provide a patient eligibility case report form for this evaluation.

4.3 Informed Consent - Signed informed consent for enrollment in this protocol will be obtained from eligible patients by the attending physician before the start of treatment. At the preadmission consultation, patients will be fully informed of the purpose and potential risks and benefits of participating in the study. Patients have the opportunity to have questions answered to their satisfaction before signing the consent.

4.4 Eligible patients must be registered Monday through Friday between 8:00 a.m. and 4:30 p.m. (Central Time) by calling Pamela Zehr, RN or Carlene Etscheidt, RN the University of Iowa Clinical Cancer Center, Iowa City, Iowa, 319-353-8914 or 319-356-1228, respectively. Information from the eligibility form will be provided by the investigator or the investigator's research staff to the University of Iowa Cancer Center at this time, and the patient will be registered and assigned a unique patient number.

4.5 No patient may be enrolled or begin treatment prior to registration and assignment of a patient number. As a follow-up, University of Iowa Cancer Center will provide the investigator with written confirmation of each patient's registration.

4.6 All investigators will be notified by the Chair of the Protocol Review and Monitoring Committee or by the trial's Data and Safety Monitoring Board if the study is placed on administrative hold, and when the study is completed or closed to further patient enrollment.

4.7 Patients must begin the vaccine protocol within 7 days of registration.

## 5. TREATMENT PLAN

### 5.1 Administration Schedule

#### Ad/PSA

Patients with recurrent disease after surgical treatment or radiation therapy will be randomized to either Arm A (vaccine only) or Arm B (androgen depletion therapy plus vaccine). All patients will receive three injections of 0.125 ml. of the Ad/PSA subcutaneously in the right thigh. The dose of the vaccine, based upon our results from the Phase I trial, will be  $1 \times 10^8$  pfu in the Gelfoam matrix. The Gelfoam comes in sterile patient-ready packages. The virus will be suspended in sterile saline and the Gelfoam powder added in a ratio of 30 mg of powder per ml. of virus suspension. Injections will be spaced apart by 30 days, such that each patient will receive the vaccine on days 0, 30, and 60. The use of the matrix has been shown in collaborative pre-clinical experiments to enhance infection of host cells by the virus. Results from the Phase I trial indicated that the injection of the vaccine in Gelfoam did not produce any adverse events greater than those produced by the vaccine in an aqueous suspension. The vaccine induced anti-PSA immune response in patients injected as an aqueous or Gelfoam vaccination. Injections will be carried out in the University of Iowa General Clinical Research Center (GCRC). Each subject will be housed in the GCRC for 24 hours and observed for early signs of toxicities. Tests, indicated in the table on page 17, will be carried out to be certain that no serious side effects are induced by the vaccine.

## 6. Adverse Events

### 6.1 Definitions

**Adverse Event (AE)** is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease\* temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

This will also include intercurrent diseases and accidents observed during treatment period as well as corresponding events during drug-free, pre- and post-treatment periods, under placebo or in a reference group receiving drug or non-drug therapy.

**Serious adverse event (SAE)** is any untoward medical occurrence that:

- a. results in death
- b. is life-threatening<sup>i</sup>

- c. requires inpatient hospitalization or prolongation of existing hospitalization
- d. results in persistent or significant disability or incapacity
- e. is a congenital anomaly / birth defect or
- f. is another medically important condition.<sup>ii</sup>

## 6.2 Procedures of documentation of AEs

All AEs occurring during the study must be documented, regardless of the assumption of a causal relationship, on the respective AE CRF. All events, which occurred after signed informed consent, should be documented. The investigator should ensure that all events are recorded that occurred within at least 4 weeks after the last exposure to the study drug.

Documentation of AEs includes: date of onset and offset, intensity, frequency, seriousness, related interventions and outcome. The investigator will also evaluate the probability of a causal relationship of the adverse event to the study medication as being: “definite, probable, possible, unlikely, or unrelated.”

### Expedited reporting

The investigator must immediately report serious adverse events (SAE) occurring or observed during the course of the study and within 4 weeks of last administration of the study drug to the NCI and institutional IRB;

After notifying the NCI by telephone of an SAE within 24 hours of the knowledge of the event’s occurrence, the “Serious Adverse Event Report” must also be sent by fax to the NCI whether or not complete information is available at the time. If complete information is unavailable the investigator must provide follow-up information to the NCI as soon as it is known.

In particular, the investigator must inform the NCI by phone and fax within 24 hours of occurrence of immediately life-threatening SAEs or SAEs with fatal outcome. SAEs must be reported to the site’s IRB according to the IRB’s requirements.

**Important: The investigator must report any SAE to the NCI, and to the IRB regardless of causality.**

Reports will be evaluated by the Medical Monitor/Sponsor. FDA/HPB and investigators will be informed as required by the regulations. The same information will also be made available to all participating investigators as well as to other investigators participating in different clinical trials utilizing the same study medication.

## 7 MEASUREMENT OF CLINICAL AND IMMUNOLOGICAL EFFECT

**7.1 Methods of Malignant Disease Evaluation** - Each patient will have a baseline evaluation prior to the injection of the Ad/PSA vaccine. The measurements will include temperature, weight, serum PSA, blood chemistries, a quantitative bone scan for bone metastases, and CT for soft tissue metastases, and performance status for quality of life.

Patients will be seen in the GCRC (see Table 5 for schedule). The injection site will be examined for evidence of erythema, induration and necrosis and patients will have their temperature and weight recorded and interviewed to determine whether they

experienced any adverse reactions. Blood samples will also be taken for measurement of PSA and anti-PSA antibodies (see Table 5). At the 6 month, 12 month, and subsequent semi-annual visits each patient will be evaluated using the measurements listed for the baseline visit.

**7.2 Use of Serum PSA for Disease Evaluation** – Based upon our pre-clinical experiments and the results from the Phase I clinical trial we expect the immunized men to produce anti-PSA antibodies. The levels of antibody will be measured by a flow cytometry assay as described by Cavacini, et al. used in our Phase I clinical trial (39). We will also explore the use of a second serum marker for prostate cancer, hK2 in collaboration with Donald Tindall, Charles Young, and George Clee at the Mayo Clinic. Investigators at Mayo, along with Hybritech, Inc. have been exploring hK2 and published a number of papers in recent years on the subject (70-73). Patient sera from each clinic visit will be sent to Mayo where they will measure the levels of hK2. We will use the data to evaluate the effect of anti-PSA antibodies on both PSA and hK2 in the sera of vaccinated patients.

### **7.3 Experimental Evaluation of the Ad/PSA Vaccination**

7.3.1 Blood will be collected prior to, and at each visit after, the injection of the Ad/PSA vaccine. Two separate samples will be collected; one in red top tubes to allow collection of serum from coagulated blood and a second in heparinized tubes to permit collection of lymphocytes.

7.3.2 Levels of PSA, hK2, anti-PSA antibodies, and anti-adenovirus antibodies will be measured in the serum.

7.3.3 Anti-PSA T cell immune responses will be measured by ELISPOT analysis using the methods developed for, and used in, our Phase I clinical trial. In addition to measuring the anti-PSA T cell activity, we will also measure anti-adenovirus T cell activity as well as reactivity to stimulation with cytomegalovirus (CMV). A non-specific stimulus will be provided by PMA and ionomycin for each patient's lymphocytes.

### **7.4 Definitions of Response** –

7.4.1 Arm A – Hormone Naïve –

#### **7.4.1.1 Primary Endpoint - Development of Anti-PSA Immune Responses**

7.4.1.1.1 Immunologic response – Definition: an increase of  $\geq 200\%$  above re-immunization levels of anti-PSA T cells as measured by ELISPOT analysis, measured at any point after vaccination.

#### **7.4.1.2 Secondary Endpoints – PSA Responses**

7.4.1.2.1 PSA response - Definition: a 50% reduction in the pre-treatment PSA value, verified with a second measurement 30 days later

7.4.1.2.2 A 50% increase in the PSADT compared to pre-enrollment PSADT.

7.4.1.2.3 PSADT will be calculated based on the MSKCC online calculator, available at <http://www.mskcc.org/mskcc/html/10088.cfm>.

7.4.1.2.4 PSADT response will be measured at 9 and 18 months after initiation of study treatment.

7.4.1.2.5 Three measurements of PSA, spaced at least 2 weeks apart, will be required prior to study enrollment. Post-treatment PSADT will be based on PSA levels at 3, 6 and 9 months (9 month PSADT calculation) and 3,6, 9,12,15,18 month levels (18 month PSADT calculation).

#### 7.4.2 Arm B – Androgen-Deprivation Therapy –

##### 7.4.2.1 Primary Endpoint - Immunologic response

7.4.2.1.1 Definition: an increase of  $\geq 200\%$  above re-immunization levels of anti-PSA T cells as measured by ELISPOT analysis, measured at any point after vaccination.

7.4.2.2 Secondary Endpoint - PSA response - Definition: a 50% reduction in the pre-treatment PSA value, verified with a second measurement 30 days later

#### 7.5 Definition of Progression

7.5.1 Development of positive bone scan (bone scans will be performed every 6 months)<sup>A</sup>

7.5.2 Development of rising PSA after nadir, if existent

### 8 STUDY PARAMETERS

8.1 Scans or x-rays used to document measurable or evaluable disease should be done with 4 weeks prior to study entry

8.2 CBC with differential, LFT's should be done  $\leq 2$  weeks before study entry.

8.3 All chemistries should be done  $\leq 2$  weeks before the study entry, unless specifically required on day 1 as per protocol. If abnormal, they must be repeated within 48 hours prior to study entry.

8.4 Hgb, Hct, WBC, Plt should be done  $\leq 2$  weeks before study entry but, if abnormal, they must be repeated <48 hours prior to study entry.

8.5 REMOVAL OF PATIENTS FROM STUDY (Criteria for discontinuation of a patient's study participation)

8.5.1 Adverse events: In the event of a vaccine-associated unmanageable or irreversible toxicity, the investigator will withdraw a patient from further treatment

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<sup>A</sup> Only patients with negative bone scans are eligible for the study

and notify the Study Chair immediately. *In addition, the FDA and the IRB will be notified of the adverse events.*

- 8.5.2 Disease Progression: Patients will be taken off-study if they have progressive disease (PD) or clinically significant deterioration at any time during the study if the investigator feels that (a) alternative prostate cancer therapy might benefit the patient, or (b) to continue on study might be unsafe for the patient. Patients receiving alternative prostate cancer therapies will still be followed for toxicity and immunologic evaluations.
- 8.5.3 Allergic Reactions: Patients will be removed from the study should they develop grade II allergic reactions.
- 8.5.4 Personal Reasons: As stated in the informed consent, patients may withdraw from the study at any time.
- 8.5.5 Clinical Judgment: A patient may be withdrawn from the study, if, in the opinion of the investigators, it is not in the patient's best interest to continue (e.g. an adverse experience, intercurrent illness, etc.)
- 8.5.6 The date of discontinuation and the reason(s) for patient discontinuation from the study will be recorded in the CRF. All evaluations that are required at the follow-up must be conducted for each patient who discontinues treatment, regardless of the reason.

### **Regulatory and Reporting Requirements**

The Data and Safety Monitoring Committee (DSMC) of the Holden Comprehensive Cancer Center will provide data and safety monitoring for this study. "The Data and Safety Monitoring Plan of the Holden Comprehensive Cancer Center" provides standard operating procedures to monitor all clinical cancer trials at the UIHC. All investigator-initiated trials are automatically monitored by the DSMC. A detailed data and safety monitoring plan for this study is on file with the DSMC and the Clinical Research Safety Officer (CRSO).

### **Data Management, Quality Control and Data Security**

In order to protect confidentiality the subject will be assigned an identification number. This number will be used on all specimens from the subject and will be used for documentation purposes.

Data management for the optimal entry, processing, storage, and retrieval for this protocol's data will be accomplished by the principal investigator. The database will be located on a computer or in a locked cabinet in a locked office. This computer will be secured, accessible only by the research team. There will be more than one copy of the database. The second, secured, copy of the protocol data will be stored in a locked room accessible only by the research team. For quality control, auditing, and checking data for integrity, there will be a regular accounting of data periodically performed

**Table 5**  
**Study Design and Testing**

	<b>Prior to Study Entry</b>	<b>At 1<sup>st</sup> Ad/PSA injection</b>	<b><i>During the first 24 hours</i></b>	<b>30 d.</b>	<b>44 d.</b>	<b>60 d.</b>	<b>74 d.</b>	<b>90 d.</b>	<b>6 mo</b>	<b>9 mo</b>	<b>12 mo</b>	<b>Every 6 mo. to prog.</b>
Physical Examination	X	X	x	X	X	X	X	X	X	X	X	X
Performance Status	X			X	X	X	X	X	X	X	X	X
Vital Signs	X	X	x	X	X	X	X	X	X	X	X	X
Weight	X	X		X	X	X	X	X	X	X	X	X
Blood for PSA, PAP	X	X		X	X	X	X	X	X	X	X	X
anti-PSA Ab, and lymphocytes for cellular immunity	X	X		X	X	X	X	X	X	X	X	X
Hgb/Hct, WBC, platelets	X	X	x	X	X	X	X	X	X	X	X	X
Differential	X		x	X	X	X	X	X	X	X	X	X
<i>AST, ALT, LDH, alkaline phosphatase, bilirubin</i>	X		x	X	X	X	X	X	X	X	X	X
Creatinine	X		x	X	X	X	X	X	X	X	X	X
Urinalysis	X		x	X	X	X	X	X	X	X	X	X
Chest x-ray	X			X		X		X	X	X	X	X
Bone scan	X								X		X	X
Abdominal/pelvic CT	X								X		X	X
EKG	X								X		X	X



## 9 DRUG FORMULATION AND PROCUREMENT

### 9.1 Drug Name

Adenovirus/PSA (Ad/PSA)

### 9.2 Classification

Vaccine

### 9.3 Mode of Action

The adenovirus is a replication-deficient virus unable to produce virus progeny in the infected cells. The virus will infect cells in the location of the injection site, the PSA gene will produce the protein product which will be recognized as an antigen by the immune system and produce anti-PSA immune responses. Based upon our pre-clinical studies in an animal model of human prostate cancer, these responses, mainly the CD8+ CTL response, will cause the destruction of PSA-secreting prostate tumors.

### 9.4 Dose Specifics and Route of Administration

The route of injection, vehicles for the vaccine, and dose schedules have been outlined in Section 6.1 of this protocol.

### 9.5 Availability

Produced and provided by Molecular Medicine, LLC, San Diego, CA

### 9.6 Manufacturing

9.6.1 The PSA cDNA provided by Donald Tindall, Mayo Clinic, Rochester, MN, was placed 3' to the CMV promoter in a shuttle vector containing Ad5 DNA. The sequence inserted was the pre-pro form of PSA described by Lundwall (75) that encodes 262 amino acids with a predicted molecular weight of 28.8 kDa. Using methods previously described (75), the shuttle vector and E1a-E1b deletion mutant Ad5 DNA were transfected into HEK 293 cells, and recombination between the DNA species was allowed to occur. The amplification and purification of Ad/PSA was performed by the University of Iowa Gene Transfer Vector Core as previously described (76). Ad/lacZ used as a control was also obtained from the Gene Transfer Vector Core and is previously described (75).

9.6.2 The Principal Investigator provided the Ad/PSA vaccine used for the pre-clinical studies to Molecular Medicine, LLC of San Diego, CA for the production of the clinical grade product. Information on the manufacturing of the GMP Vaccine by Molecular Medicine, LLC is found in the accompanying documents supplied by the company.

## 10 STATISTICAL CONSIDERATIONS

10.1 **Statistics** - The ideal endpoint would be a clinical outcome that is of particular relevance to the patient such as increased time to tumor progression, increased time

to death or reducing the proportion of death. This trial is using a surrogate endpoint as a substitute to the clinically meaningful outcome since the tumor cannot be accessed directly. The association between the surrogate and survival rate had not been clearly established by any phase I & II trial. The trial consists of using Ad/PSA vaccine administered in multiple injections to prostate cancer patients with minimal disease burden—with the goal to induce anti-PSA T cells responses. Three injections of equal dose are proposed. The previous phase I trial consisting of a single injection in men with metastatic prostate cancer was able to induce anti-PSA T cells responses. The Phase I consisting of a single injection using a dose escalation protocol of the vaccine in an aqueous or matrix delivery vehicle did not show any significant AE. Additional pre-clinical pharmacology /toxicology studies required by the FDA did not show any significant side effects using the three-injection schedule. The primary endpoints depend on the different treatment arms. For the vaccine-only group, the endpoint is the serum PSADT. For Vaccine & hormone group, the primary endpoint will be the categorized immune response. The reason for the differences in primary endpoints in these two patient populations is that men treated by androgen deprivation therapy will have a sharp decrease in serum PSA making it impossible to follow changes in PSADT.

- 10.2 **Endpoint based on PSADT** - For efficacy purposes, we expect at least 50% of the patients to show 50% increase in PSADT. This proportion is judged clinically important and anything less than 30% can stop the trial for futility. Thus, a total sample size of 46 patients will be needed. The ideal design would be a two-stage design of Simon (77). After testing the treatment on 15 patients, if 5 did not have a 50% increase in PSADT, the trial will be terminated. Otherwise, an additional 31 patients will be recruited for the trial. The expected sample size will be 23.63 and the probability of early termination, 0.72. If the treatment is not effective, there is 0.05 probability of concluding that it is. If the treatment is effective, there will be 0.2 probability of concluding that it is not. However, due to the complexity of the trial, we will not use Simon (77) but a more conservative approach consisting of having a one-stage design at efficacy level and a three-stage design at toxicity level. By designing the trial this way, we are making the probability of early termination for efficacy purposes to be zero. Early termination will be based on toxicity only. The reason for this design is due to the nature of the treatment and is explained below. Enrollment will not stop unless stopping rules based on toxicity are satisfied:

*“After the third vaccine is administered, toxicity will be evaluated 90 days into the trial”*

Due to the nature of the treatment, anti-tumor activity will be potentially delayed and the primary efficacy endpoint will be determined 18 to 20 months after initial patient accrual. Since we will be able to assess PSADT after nearly 20 months into the study, it will be unreasonable to stop the study due to unsatisfactory results prior to that point. This is the main reason why we will carry a one-stage design for the primary endpoint and a three-stage design for toxicity. Criteria for proceeding with enrollment into a subsequent stage prior to the two-year efficacy evaluation will be based on assessment of toxicity in the patient cohorts since this can be done as early as 90 days into the trial. The optimal three-stage design to test the null hypothesis that the toxicity level is less than 15% versus the alternative that it is greater than 35% has an expected sample size of 22, a probability of early termination after the first stage of 0.49, and a probability of early termination after the second stage of 0.78. If the toxicity level is high, there will be a 0.05 probability of concluding that it is not. If the

level is actually low, there is a 0.1 probability of concluding that it is not. After enrolling 10 patients in the first stage, the trial will be terminated if 4 or higher show grade 3 toxicity or higher. After testing the drug on 23 patients in the first and second stages, the trial will be terminated if 7 or more show grade 3 toxicity or higher. After testing 46 patients in all three stages, the trial will be terminated if 12 or higher show grade 3 toxicity or higher. This corresponds to testing the hypothesis that  $p_0 > 35\%$  versus  $p_1 < 15\%$  sequentially and in three stages at a fixed significance level of 0.05.

- 10.3 **Endpoint based on Immune response** - The primary endpoint for patients receiving the vaccine plus androgen deprivation therapy is the development of anti-PSA immune responses. In the Phase I trial of the vaccine alone, patients that developed anti-PSA T cell responses had an increase in the frequency of anti-PSA T cells. We decided to express the anti-T cell response as percent change in antigen-specific T cell frequency compared to the frequency prior to immunization. Based on our phase I results, we judge 70% of the patients developing anti-PSA immune response as being clinically important while anything less than 40% will be judged unworthy. For the same reasons as we stated earlier, we will conduct a one-stage design for efficacy and a three-stage design for toxicity. A total of 23 patients will be needed. If 13 or fewer show immune response, the vaccine will be judged unworthy for further consideration. If the vaccine is actually not effective, there is a 0.05 probability of concluding that it is. If the vaccines are actually effective, there will be a 0.12 probability of concluding that it is not. Note thought, that we have made the probability of early termination to be zero so as to base termination on toxicity only.

The optimal three-stage design to test the null hypothesis that the toxicity level is less than 15% versus the alternative that it is greater than 35% has an expected sample size of 9, a probability of early termination after the first stage of 0.44, and a probability of early termination after the second stage of 0.83. If the toxicity level is high, there will be a 0.05 probability of concluding that it is not. If the level is actually low, there is a 0.2 probability of concluding that it is not. After the enrollment of 2 patients in the first stage, the trial will be terminated if 1 or both patients show grade 3 toxicity or higher. After testing the vaccine on 11 patients in the first and second stages, the trial will be terminated if 2 or more show grade 3 toxicity or higher. After testing 22 patients in all three stages, the trial will be terminated if 3 or higher show grade 3 toxicity or higher. The reasons for using a three-stage design instead of two-stage or one-stage are because we are using surrogate endpoints that have not been proved formerly to have an ease of predictability of an outcome of direct relevance to patients; also, it is not quite clear how these surrogates relate to the pathway of the natural disease and to overall survival rate.

- 10.4 **Randomization to clinical arm:** two-to-one.

- 10.5 **Reference Sources for Statistical Considerations**

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<sup>i</sup> The term “life-threatening” in the definition of “serious” refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe.

<sup>ii</sup> Medically important conditions that may not result in death, be life-threatening or require hospitalization may be considered as SAE when, based upon appropriate medical judgment, they may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse. N.B.: The term “severe” is often used to describe the intensity (severity) of an event (such as: mild, moderate, or severe e.g., pain). The event itself may be of relatively minor medical significance (such as severe headache). This is not the same as “serious”, which is based on patient/event outcome or action criteria usually associated with events that pose a threat to patient’s life or vital functions. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.



**PHASE II STUDY OF ADENOVIRUS/PSA VACCINE IN MEN WITH  
HORMONE - REFRACTORY PROSTATE CANCER**

Version 7 – June 7, 2006

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## 1. INTRODUCTION

### 1.1 Background- Immunotherapy in Prostate Cancer

Prostate cancer is the second leading cause of cancer death among males in the United States. There will be an estimated 234,460 new diagnoses of prostate cancer made in the United States in 2006 (1). Treatments for organ-confined prostate cancer include radical prostatectomy and radiation therapy. When the cancer presents de novo, or recurs outside the prostate, first-line systemic treatments typically include hormonal blockade (with LHRH agonists or bilateral orchiectomy), which suppress testosterone levels, limit the growth of androgen-dependent cancer cells, and result in clinical tumor control. After a median time of 2 years, patients progress into a clinical hormone-refractory state, when the prostate specific antigen (PSA) levels rise despite castration, there is proliferation of androgen-independent cancer cells, and there is continued clinical tumor growth that becomes fatal. Therapeutic measures in this situation include further hormonal manipulations or the use of systemic chemotherapy, which has recently shown a small survival benefit in phase III trials. Approximately 30,000 Americans die from prostate cancer each year.

Immunotherapeutic approaches against prostate cancer have been investigated for several years. Most of these studies have concentrated on active non-specific therapy and adoptive or passive therapy, with only recent focus on the induction of antigen-specific immune responses. Viral vectors have been used successfully in both gene transfer and vaccine therapy studies (2). Replication-competent and replication-deficient adenoviruses expressing foreign proteins have been used to elicit immune responses to a variety of tumor antigens (3-7).

We have demonstrated that immunizations with adenovirus, carrying the human PSA gene, can induce vigorous anti-PSA T-cell responses and cause the destruction of PSA-secreting tumors in a pre-clinical mouse model of prostate cancer (8,9). Such active immunization against prostate-cancer associated antigens might be more effective than active non-specific or adoptive/passive immunotherapy. Therefore, we have pursued a vaccination strategy based on an adenovirus that carries the gene for prostate specific antigen (PSA). Results from our Phase I trial of adenovirus/PSA (Ad/PSA) vaccine (section 1.4, below) demonstrated that a single immunization of men with metastatic prostate cancer was able to induce anti-PSA T cell responses. The trial design was a dose escalation study with the vaccine administered subcutaneously (sc) either in an aqueous solution or in a collagen matrix (Gelfoam®). We now propose a Phase II clinical trial using the Ad/PSA vaccine, administered in multiple injections to prostate cancer patients with minimal disease burden.

### 1.2 Adenovirus vectors

Recombinant adenoviral vectors transduce a wide range of dividing and nondividing cells types, making this gene delivery system valuable as a tool for studying diseases, for vaccine therapy, and for potential clinical use (10). Recombinant adenovirus can be prepared and purified in high titers. In addition, wild-type adenovirus infections are extremely common in the general population, giving adenovirus a well-documented safety record (11). Moreover, adenoviruses are structurally stable and no adverse effects have been reported following the vaccination of US military recruits with wild types, demonstrating their safety for human use (11). Adenoviral vectors for gene therapy and vaccine therapy are adenoviruses which have been genetically modified to allow insertion of foreign genes and to render the virus replication-defective. Current vectors have a deletion in the E1 region or in both the E1 and E2 regions.

Adenoviral gene transfer has been used in a variety of experimental conditions that include transfers to the liver (12), lung (13), central nervous system (14,15), and to cancer cells (16).

There is evidence that the introduction of foreign transgenes by adenovirus induces immune responses to the transgene product, which become ultimately responsible for the elimination of the virus (17,18). While this is disadvantageous for insertion of functional genes into host cells, it is advantageous in the use of viruses carrying foreign genes as immunogens. In the vaccine therapy of cancer, active immunization against a murine colon cancer, breast cancer, and melanoma antigens have been induced by adenoviral vaccines (19-26).

The Ad/PSA vaccine used our laboratory and in our Phase I clinical trial was produced by inserting the gene for the full length pre-pro form of human PSA into a replication deficient adenovirus serotype 5. Replication deficiency was induced by deletion of the E1a and E1b genes of the virus. Details of the vaccine can be found in section 9 of this protocol. Approval for the use of the vaccine in the Phase I trial was obtained from the FDA under IND #9706.

In pre-clinical studies, our group has demonstrated that the Ad/PSA vaccine was able to induce stronger anti-PSA immune responses than other viral PSA vaccines. These include vaccinia viruses, both replication competent and replication deficient, and to a canarypox vaccine (Table 1). The frequency of PSA-specific CD8+ cells T cells generated by the Ad/PSA vaccine was greater than were generated by any of the other vaccines tested.. In addition to the superior immunizing property of the Ad/PSA, the incorporation of Gelfoam, a collagen matrix (section 1.3), has been shown in pre-clinical studies to enhance the ability of the vaccine to induce strong anti-PSA immune responses (8). Lastly, immunization of mice with Ad/PSA in matrix can induce anti-PSA responses even in the presence of high titer anti-adenovirus antibodies (8). This latter finding is important in light of the fact that most humans have pre-existing levels of anti-adenovirus antibodies as a result of prior natural exposure to the virus.

**Table 1**  
**Effector Cell Frequency Analysis (ELISPOT)**

<b>Vaccine</b>	<b>Virus</b>	<b>Frequency of PSA-Specific CD8+ T Cells</b>
Ad/PSA*	Replication deficient adenovirus	1/455
Prostvac	Replication competent vaccinia	1/2028
NYVAC/PSA	Replication deficient vaccinia	1/3597
ALVAC/PSA	Canarypox	1/35,714

### 1.3 Gelfoam<sup>®</sup> Matrix

Gelfoam (Pharmacia & Upjohn Company, Kalamazoo, MI) is a medical device intended for application to bleeding surfaces as a hemostatic agent. It is a water-insoluble, off-white, non-elastic, porous, pliable product prepared from purified pork skin. The Gelfoam gelatin preparation is available either as a cross-linked sponge or as non-cross linked beads. It is able to absorb and hold within its interstices approximately 45 times its weight of blood and other fluids (26). The absorptive capacity of Gelfoam is a function of its physical size, increasing with increasing gelatin volume (27).

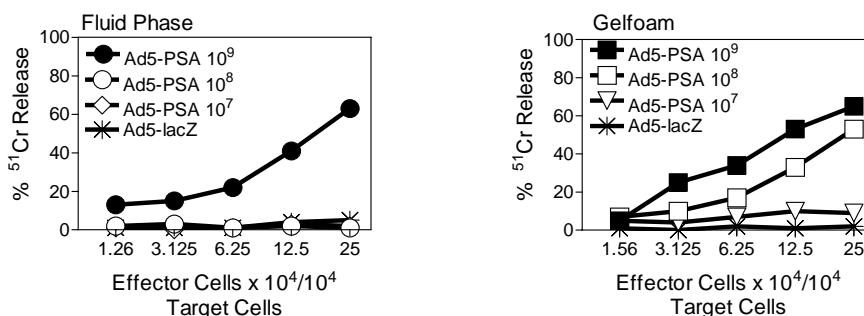
The mechanism of action of surface-mediated hemostatic devices is supportive and mechanical (27). Surface-acting devices, when applied directly to bleeding surfaces, arrest bleeding by the formation of an artificial clot and by producing a mechanical matrix that facilitates clotting (28). Jenkins et al have theorized that the clotting effect of Gelfoam may be due to release of thromboplastin from platelets, occurring when platelets entering the Gelfoam become damaged by contact with its myriad of interstices (29). Thromboplastin interacts with prothrombin and calcium to produce thrombin, and this sequence of events initiates the clotting reaction. The authors suggest that the physiologic formation of thrombin in Gelfoam is sufficient to produce formation of a clot, by its action on the fibrinogen in blood (29). The spongy physical properties of Gelfoam hasten clot formation and provide structural support for the forming clot (28,30).

Gelfoam has been used experimentally for the delivery of soluble proteins and drugs, including insulin, antibiotics, and growth factors (31-33). Gelfoam was used for sustained release of insulin in an ocular implant device (31). Delivery of insulin in solution had no effect on blood glucose levels. In contrast, the use of Gelfoam as a sustained release delivery agent provided measurable insulin activity for up to 10 hours after implantation. Glucose levels in the blood stabilized at 60% of the original value, whereas administration of insulin in eye drops had no effect.

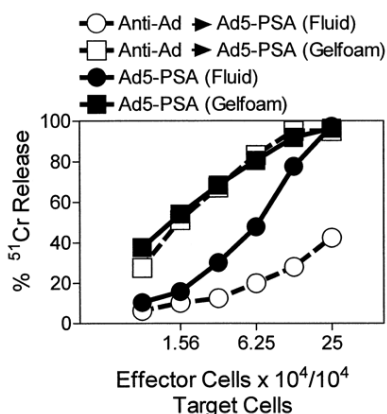
MacDonald and Mathews (34) studied Gelfoam implants in canine kidneys and reported that it assisted in healing, with no marked inflammatory or foreign-body reactions. Jenkins and Janda (35) studied the use of Gelfoam in canine liver resections and noted that Gelfoam appeared to offer a protective cover and provide structural support for the reparative process. Correll et al (36) studied the histology of Gelfoam when implanted in rat muscle and reported no significant tissue reaction.

Gelfoam has been used as a hemostatic agent in dog prostate (37). In these studies no gross histological evidence of tissue damage or calcification was induced. In addition, these investigators demonstrated that placement of Gelfoam into the lumen of the bladder resulted in liquefaction of the Gelfoam without any evidence of calculogenesis. Finally, Bischoff and Goertler (38) used Gelfoam in human prostate therapeutic embolization with success.

Our laboratory, in collaboration with Dr. Timothy Ratliff, has demonstrated that administration of the Ad/PSA vaccine in Gelfoam induces a stronger anti-PSA immune response (Figure 1). In our pre-clinical studies, immunization with the vaccine in an aqueous suspension induces strong immunity with  $10^9$  pfu with weaker immunity induced with  $10^8$  and  $10^7$  pfu. Use of Gelfoam permits the induction of strong responses at the lower dose of  $10^8$  pfu. In addition, strong anti-PSA T cell responses could be induced by immunization with the Ad/PSA vaccine in Gelfoam even in mice pre-immunized to adenovirus (Figure 2). In the Phase I clinical trial (section 1.4), the addition of Gelfoam to the vaccine immunization did not result in excess serious adverse events.



**Figure 1** – Anti-PSA cytotoxic activity of spleen cells obtained from mice immunized with Ad/PSA in aqueous (fluid phase) versus Gelfoam.



**Figure 2.** Effect of the presence of anti-adenovirus Ab on Ad5-PSA immunization with and without Gelfoam. Mice were injected i.p. with  $10^8$  PFU of Ad5-lacZ or PBS 2 wk before immunization with  $10^8$  PFU Ad5-PSA delivered s.c. in the fluid phase (PBS) or Gelfoam. CTL were tested against RM-11psa and RM-11neo to demonstrate PSA specificity. Cytolytic activity of each group against RM-11neo targets was  $<10\%$  for all E:T ratios.

#### 1.4 Phase I study

A Phase I clinical trial of the Ad/PSA vaccine has been completed in men with measurable metastatic prostate cancer, with the primary objectives of determining the toxicity profile and maximal tolerated dose (MTD). The ability of the vaccine to induce anti-PSA immune responses and any clinical responses was also evaluated.

Eligible patients consisted of men with prostate cancer that had measurable metastatic disease, 90% of whom were stage D3. Prior therapies had included androgen depletion, ketaconazole, prednisone, Casodex, Taxotere, and external beam radiation, but the initiation of vaccine therapy was equal to, or greater than, 30 days after the most recent therapy. Patients were treated in successive dose levels and aqueous vs. matrix cohorts, according to the protocol plan. We were able to administer the maximum permitted dose of  $10^8$  pfu without any serious adverse events by treatment of the first of 18 patients. These initial 18 patients were followed throughout the one-year period after injection. An additional 14 patients were treated at the MTD dose level, as planned in the protocol and confirmed by a letter to the FDA. The purpose of the additional patients was to have sufficient numbers of patients in the groups to statistically evaluate the anti-PSA immune responses induced by the Ad/PSA vaccine. In summary, 32 patients were treated in the study followed through the one-year period. Two additional patients were enrolled in the study (total number of enrolled patients, 34) but never received the vaccine and were therefore not evaluable. One patient chose to have radiation therapy instead of participating in the trial and the second was diagnosed with a second malignancy (melanoma) shortly after his enrollment in the phase I study.

##### 1.4.1 Phase I study results

The median age of the patients was 70.2 years (range, 52 to 89). The vaccine was administered as an aqueous suspension or in a collagen (Gelfoam) matrix to 32 patients. Sixteen (16/32) or 50% of the patients exhibited grade 1 vaccine-related adverse events (AE), 1/32 (3.1%) that exhibited a grade 2 AE, and one patient exhibited a grade 3 AE which was a decrease in neutrophil count. There were no vaccine-related grades 4 or 5 AEs.

We measured the anti-PSA immune responses, both antibody and T cell, in all patients enrolled in the study. Antibody responses to PSA were measured by the binding to PSA-secreting cell lines using the method adapted from Cavacini, et al. (39). Results of those analyses demonstrated that 57% of men immunized with the Ad/PSA vaccine developed measurable anti-PSA antibodies. ELISPOT assays were utilized to measure anti-PSA T cell

responses. The results, depicted in Table 2, demonstrate that of the 32 patients, 18 (56.3%) developed anti-PSA T cell responses. The addition of Gelfoam did not appear to affect the development of anti-PSA responses, but in this Phase I study the numbers of patients in each group was too small to make statements of statistical significance of the data. These results demonstrate the ability of men with late stage metastatic prostate cancer, injected one time with Ad/PSA, to respond to the vaccine with the production of anti-PSA T cells.

**Table 2**  
**ELISPOT Analysis of Anti-PSA T Cell Immune Responses**

Patient Number	Dose/ Vehicle	Response	Frequency	
			Pre-Immunization	Post- Immunization
AP-002	10 <sup>6</sup> -aqueous	-	1/985,000	1/258,571
AP-004	10 <sup>6</sup> -aqueous	-	0	1/311,111
AP-007	10 <sup>6</sup> -aqueous	+	1/46,901	1/14,804
AP-003	10 <sup>6</sup> -matrix	+	1/90,000	1/8075
AP-005	10 <sup>6</sup> -matrix	+	1/1.2x10 <sup>6</sup>	1/152,353
AP-006	10 <sup>6</sup> -matrix	+	1/93,103	1/15,762
AP-008	10 <sup>7</sup> -aqueous	-	1/100,000	1/97,419
AP-010	10 <sup>7</sup> -aqueous	-	0	0
AP-013	10 <sup>7</sup> -aqueous	+	0	1/60,000
AP-009	10 <sup>7</sup> -matrix	+	1/52,535	1/30,566
AP-012	10 <sup>7</sup> -matrix	-	1/13,488	1/254,286
AP-014	10 <sup>7</sup> -matrix	+	1/562,500	1/120,000
AP-015	10 <sup>8</sup> -aqueous	+	1/1.4x10 <sup>6</sup>	1/780
AP-016	10 <sup>8</sup> -aqueous	+	0	1/130,000
AP-017	10 <sup>8</sup> -aqueous	+	0	1/124,375
AP-025	10 <sup>8</sup> -aqueous	-	0	0
AP-026	10 <sup>8</sup> -aqueous	-	0	1/560,000
AP-027	10 <sup>8</sup> -aqueous	+	0	1/26,364
AP-029	10 <sup>8</sup> -aqueous	+	0	1/333
AP-032	10 <sup>8</sup> -aqueous	+	1/250,000	1/3793
AP-018	10 <sup>8</sup> -matrix	-	0	0
AP-019	10 <sup>8</sup> -matrix	+	1/14,235	1/336
AP-020	10 <sup>8</sup> -matrix	+	1/8824	1/1802
AP-021	10 <sup>8</sup> -matrix	+	1/689	1/431
AP-022	10 <sup>8</sup> -matrix	+	0	1/180,000
AP-023	10 <sup>8</sup> -matrix	-	1/320,000	0
AP-030	10 <sup>8</sup> -matrix	+	1/666.667	1/32,000
AP-034	10 <sup>8</sup> -matrix	+	1/80,000	1/15,152
AP-035	10 <sup>8</sup> -matrix	+	1/5295	1/2459
AP-036	10 <sup>8</sup> -matrix	+	1/62,000	1/9487
AP-037	10 <sup>8</sup> matrix	-	1/2.1x10 <sup>6</sup>	1/965,000

The effects of vaccination on serum PSA and on patient survival were also evaluated as a secondary endpoint in the phase I trial. Although there was no sustained decline in individual PSA levels, the PSA doubling times (PSADT, calculated based on 3 pre-enrollment consecutive PSA measurements) were reduced in 54 percent of the patients compared to pre-vaccine administration, with the best responses occurring in patients immunized with the highest dose of the vaccine (Table 3). In addition, published survival nomograms for patients with hormone refractory prostate cancer were applied to patients in this phase I trial (40,41). Table 4 shows that 57% of all patients at all doses, whether injected with the vaccine as an aqueous

suspension or in the collagen matrix, had a survival time longer than that predicted by the nomogram, The range of increased survivals in the different groups was 33% to 100%.

**Table 3**  
**PSA Doubling Time (DT) in Phase I Patients**

<b>Vaccine Dose &amp; Vehicle</b>	<b>Percent iNcreased PSA DT</b>
10 <sup>6</sup> aqueous	33%
10 <sup>6</sup> matrix	33%
10 <sup>7</sup> aqueous	67%
10 <sup>7</sup> matrix	67%
10 <sup>8</sup> aqueous	25%
10 <sup>8</sup> matrix	62%
<b>Total</b>	<b>54%</b>

**Table 4**  
**Ad/PSA Phase I Trial**  
**Predicted and Actual Patient Survival**

<b>Vaccine Dose</b>	<b>Vehicle</b>	<b>Percent with Longer Than Predicted Survival</b>
10 <sup>6</sup>	Aqueous	33%
10 <sup>6</sup>	Matrix	67%
10 <sup>7</sup>	Aqueous	100%
10 <sup>7</sup>	Matrix	33%
10 <sup>8</sup>	Aqueous	56%
10 <sup>8</sup>	Matrix	50%
<b>Overall</b>		<b>57%</b>

### 1.5 Other immunotherapy clinical trials for prostate cancer

The last several years have seen an increase in the number of clinical trials using vaccine immunotherapy for the treatment of prostate cancer. The trials have used a variety of target antigens that have been shown to be associated with prostate and prostate cancer cells. These include PSA (39,42-49), prostatic acid phosphatase (PAP) (50-53), prostate specific membrane antigen (PSMA) (54-56), telomerase (hTERT) (57,58), Thomsen-Friedenreich antigens (59), mucins (60), carbohydrates (61), and HLA-associated peptides (62). A variety of vectors have been used in the immunization process that include dendritic cells (45,50-58,63), vaccinia virus (39,42,43,47,49), fowlpox virus (39,47), liposomes (44), plasmids, (48), and chemical conjugates (59-61).

Recently, Kaufman et al. recently reported the results of a phase II trial (ECOG 7897) with a prime/boost vaccine using vaccinia virus and fowlpox virus expressing human PSA in patients with hormone-dependent prostate cancer (64). Sixty-four eligible patients with biochemical progression after local therapy were randomly assigned to three treatment arms: (A) fowlpox-PSA (rF-PSA) by intramuscular injection every six weeks for four doses, (B) rF-PSA for three doses followed by vaccinia-PSA (rV-PSA) given by intradermal injection, or (C) rV-PSA followed by three rF-PSA vaccines. Dreicer et al. reported a randomized phase II study with a recombinant Modified Vaccinia Ankara virus which expresses both MUC1 and IL2 (TG4010) (65). MUC1 is a glycoprotein associated with several malignancies. Eligible patients were



required to have no evidence of metastatic disease following curative intent local therapy, evidence of PSA failure (PSA over 2 ng/ml) and PSA doubling time (PSA-DT) less than 10 months. Arm 1 had TG4010 injected sc weekly, at a dose of  $10^8$  pfu, for six weeks, then every three weeks. Arm 2 had  $10^8$  pfu TG4010 injected sc every three weeks. Therapy was continued to disease progression or to a maximum of 36 weeks. Lastly, Small et al. recently presented the results of a phase III trial with APC8015, an immunotherapy cellular product consisting of autologous peripheral blood mononuclear cells enriched for a dendritic cell fraction pulsed with PA2024, a Prostatic Acid Phosphatase (PAP)-GM-CSF construct (66). Patients with asymptomatic, metastatic hormone-refractory prostate cancer were randomized (2:1) to receive APC8015 (n=82) or placebo (n=45) every 2 weeks x 3.

ECOG is currently planning a phase III trial using the Vaccinia virus (PROSTVAC-V/TRICOM) followed by Folwlpox virus vaccination (PROSTVAC-F/TRICOM) with GM-CSF, compared with placebo vaccine plus GM-CSF in patients with hormone-refractory prostate cancer with absence of metastatic disease (ECOG 1805, PARADIGM).

In summary, the results from these trials vary in terms of patient populations studied (hormone dependent vs. independent) and in levels of positive results, which include the induction of antigen-specific immune responses, decreases in levels of serum PSA and in rates of change in PSA velocity, and measures of clinical responses. Thus far no single vaccine immunotherapy has proven to be definitely superior to others in terms of clinical benefit, and other phase II and III trials continue to be planned or conducted. The results of some of these vaccine trials raise the question that an increase in PSADT may in the future represent a possible surrogate marker for increased time to progression, or overall survival in immunotherapy studies, and that absolute PSA responses may not constitute an obligatory step for the ultimate demonstration of clinical benefit of immunotherapy approaches in prostate cancer. Furthermore, the T-cell stimulation index may have important correlation with clinical vaccine efficacy, as seen in the phase III trial by Small et al.(66). These developing notions further support the current proposal for clinical development of our Ad/PSA vaccine, also based on the results of our prior phase I trial.

#### 1.6 Proposed phase II clinical trial: rationale

Based on the significant pre-clinical activity of the Ad/PSA vaccine in generating tumor-specific T cells, and the encouraging safety and efficacy results from our phase I study, we propose to continue the clinical development of the Ad/PSA vaccine with the performance of the current phase II trial. It is our contention that the vaccine product and the method of immunization set this therapy apart from other ongoing prostate cancer investigational immunotherapeutic approaches. Specifically, the incorporation of Gelfoam, not present in other vaccine preparations, enhances the induction of strong anti-PSA responses. Immunization of mice with Ad/PSA in Gelfoam matrix was able to induce anti-PSA responses even in the presence of high-titer anti-adenovirus antibodies. Notably, most humans naturally possess high titers of anti-adenovirus antibodies due to natural exposure to adenoviruses.

We plan to enroll prostate cancer patients with hormone-refractory metastatic disease into this Phase II clinical trial. This is similar to the population that constituted the majority of patients in our Phase I toxicity trial of the Ad/PSA vaccine. Patients in this trial will have low burden of disease, despite the fact that they are hormone refractory, i.e., have negative bone scans and/or low serum PSA. We will be comparing the clinical and immunologic response of these patients with two other patient populations in the trial. The latter populations, described in a separate protocol, will be patients with newly recurrent disease, either hormone naïve or during androgen

depletion therapy. Although the patients with recent recurrences will have a smaller tumor burden than will the patients in this protocol that have metastatic disease, the observation period to detect a therapeutic effect of the vaccine will be shorter in the latter patient population than the former population. It is for this important reason that we will enroll patients with metastatic disease on this protocol. Patients will be eligible if they have recent evidence of hormone refractory disease (D3) and either (a) have a positive bone scan with a PSA doubling time of  $\geq 12$  months, a total PSA of  $< 5$  ng/ml, and asymptomatic; or (b) have a negative bone scan with any PSA doubling time, asymptomatic, and not a candidate for chemotherapy.

## 2. OBJECTIVES

### 2.1 Primary Objective

To evaluate the response rates (PSA responses and changes in PSADT) following immunization with the Ad/PSA vaccine using a prime-boost immunization strategy, in patients with hormone refractory metastatic disease.

### 2.2 Secondary objectives

2.2.1 To evaluate the development of anti-PSA immune responses in study patients.

2.2.2 To evaluate biochemical (PSA recurrence) and radiographic (bone scans) time to progression and overall survival in evaluable patients receiving the Ad/PSA vaccine.

## 3. SELECTION OF PATIENTS

As described in Section 1.6 prostate cancer patients with hormone refractory metastatic disease will be enrolled in the study.

### 3.1 Inclusion criteria:

3.1.1 Men with prostate cancer who present with evidence of hormone refractory disease (D3).

3.1.2 Men with a positive bone scan, a PSA doubling time of  $\geq 12$  months, and a total PSA of  $< 5$  ng/ml, and asymptomatic.

3.1.3 Men with a negative bone scan with any PSA doubling time, asymptomatic, and not a candidate for chemotherapy.

3.1.4 Scans must be obtained within 6 weeks of entry into the trial.

3.1.5 Written informed consent.

3.1.6 Age  $\geq 18$  years.

3.1.7 Required laboratory values (obtained within 2 weeks of study entry)

3.1.7.1 Serum creatinine  $\leq 2.0$  mg/dL

3.1.7.2 Adequate hematologic functions: granulocytes  $\geq 1800$  per  $\text{mm}^3$  and platelets  $\geq 100,000$  per  $\text{mm}^3$

3.1.7.3 Adequate hepatocellular function: AST  $<3\times$  normal and bilirubin  $<1.5$  mg/dl.

3.1.7.4 Castrate levels of testosterone of  $<5$  ng/ml.

### 3.2 Exclusion criteria:

3.2.1 Active or unresolved infection.

3.2.2 Parenteral antibiotics  $<7$  days prior to study entry.

3.2.3 Evidence of prior or current CNS metastases. Specific imaging is not necessary in the absence of signs or symptoms.

3.2.4 Co-morbid medical conditions which would result in a life expectancy (participation) of less than 1 year.

3.2.5 Patients with compromised immune systems; congenital, acquired, or drug-induced (immunosuppressive agents) will be excluded from the study. Use of prednisone at doses higher than 10 mg daily (or equipotent steroid doses) for more than 7 days within the last 3 months is not allowed.

3.2.6 No-pre-existing malignancies that required treatment within the past 5 years except for basal or squamous cell cancers of the skin.

3.2.8 Prior participation in any vaccine studies for any disease.

3.2.9 Nor prior chemotherapy. Casodex or ketoconazole treatment must have been completed at least 6 weeks prior to registration.

## 4. Registration Procedures

4.1 All patients will be registered through the Department of Urology at the University of Iowa Hospitals and Clinics.

4.2 Patients who are candidates for enrollment into the study will be evaluated for eligibility by the clinical investigators to ensure that the criteria outlined in Section 3 have been satisfied and that the patient is eligible for participation in this clinical investigation. The University of Iowa will provide a patient eligibility case report form for this evaluation.

4.3 Informed Consent - Signed informed consent for enrollment in this protocol will be obtained from eligible patients by the attending physician before the start of treatment. At the preadmission consultation, patients will be fully informed of the purpose and potential risks and benefits of participating in the study. Patients have the opportunity to have questions answered to their satisfaction before signing the consent.

4.4 Eligible patients must be registered Monday through Friday between 8:00 a.m. and 4:30 p.m. (Central Time) by calling Pamela Zehr, RN or Carlene Etscheidt, RN the University of Iowa Clinical Cancer Center, Iowa City, Iowa, 319-353-8914 or 319-356-1228, respectively. Information from the eligibility form will be provided by the investigator or the investigator's research staff to the University of Iowa Cancer Center at this time, and the patient will be registered and assigned a unique patient number.

4.5 No patient may be enrolled or begin treatment prior to registration and assignment of a patient number. As a follow-up, University of Iowa Cancer Center will provide the investigator with written confirmation of each patient's registration.

4.6 All investigators will be notified by the Chair of the Protocol Review and Monitoring Committee or by the trial's Data and Safety Monitoring Board if the study is placed on administrative hold, and when the study is completed or closed to further patient enrollment.

4.7 Patients must begin the vaccine protocol within 7 days of registration.

## 5. TREATMENT PLAN

### 5.1 Administration Schedule

#### Ad/PSA

All patients will receive three injections of 0.125 ml. of the Ad/PSA subcutaneously in the right thigh. The dose of the vaccine, based upon our results from the Phase I trial, will be  $1 \times 10^8$  pfu in the Gelfoam matrix. The Gelfoam comes in sterile patient-ready packages. The virus will be suspended in sterile saline and the Gelfoam powder added in a ratio of 30 mg of powder per ml. of virus suspension. Injections will be spaced apart by 30 days, such that each patient will receive the vaccine on days 0, 30, and 60. The use of the matrix has been shown in collaborative pre-clinical experiments to enhance infection of host cells by the virus. Results from the Phase I trial indicated that the injection of the vaccine in Gelfoam did not produce any adverse events greater than those produced by the vaccine in an aqueous suspension. The vaccine induced anti-PSA immune response in patients injected as an aqueous or Gelfoam vaccination. Injections will be carried out in the University of Iowa General Clinical Research Center (GCRC). Each subject will be housed in the CRC for 24 hours and observed for early signs of toxicities. Tests, indicated in the table on page 16, will be carried out to be certain that no serious side effects are induced by the vaccine.

## 6. Adverse Events

### 6.1 Definitions

**Adverse Event (AE)** is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease\* temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

This will also include intercurrent diseases and accidents observed during treatment period as well as corresponding events during drug-free, pre- and post-treatment periods, under placebo or in a reference group receiving drug or non-drug therapy.

**Serious adverse event (SAE)** is any untoward medical occurrence that:

- a. results in death
- b. is life-threatening<sup>A</sup>
- c. requires inpatient hospitalization or prolongation of existing hospitalization
- d. results in persistent or significant disability or incapacity
- e. is a congenital anomaly / birth defect or
- f. is another medically important condition.<sup>B</sup>

## 6.2 Procedures of documentation of AEs

All AEs occurring during the study must be documented, regardless of the assumption of a causal relationship, on the respective AE CRF. All events, which occurred after signed informed consent, should be documented. The investigator should ensure that all events are recorded that occurred within at least 4 weeks after the last exposure to the study drug.

Documentation of AEs includes: date of onset and offset, intensity, frequency, seriousness, related interventions and outcome. The investigator will also evaluate the probability of a causal relationship of the adverse event to the study medication as being: “definite, probable, possible, unlikely, or unrelated.”

### Expedited reporting

The investigator must immediately report serious adverse events (SAE) occurring or observed during the course of the study and within 4 weeks of last administration of the study drug to the NCI and institutional IRB;

After notifying the NCI by telephone of an SAE within 24 hours of the knowledge of the event's occurrence, the “Serious Adverse Event Report” must also be sent by fax to the NCI whether or not complete information is available at the time. If complete information is unavailable the investigator must provide follow-up information to the NCI as soon as it is known.

In particular, the investigator must inform the NCI by phone and fax within 24 hours of occurrence of immediately life-threatening SAEs or SAEs with fatal outcome. SAEs must be reported to the site's IRB according to the IRB's requirements.

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<sup>A</sup> The term “life-threatening” in the definition of “serious” refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe.

<sup>B</sup> Medically important conditions that may not result in death, be life-threatening or require hospitalization may be considered as SAE when, based upon appropriate medical judgment, they may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse. N.B.: The term “severe” is often used to describe the intensity (severity) of an event (such as: mild, moderate, or severe e.g., pain). The event itself may be of relatively minor medical significance (such as severe headache). This is not the same as “serious”, which is based on patient/event outcome or action criteria usually associated with events that pose a threat to patient's life or vital functions. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

**Important: The investigator must report any SAE to the NCI, and to the IRB regardless of causality.**

Reports will be evaluated by the Medical Monitor/Sponsor. FDA/HPB and investigators will be informed as required by the regulations. The same information will also be made available to all participating investigators as well as to other investigators participating in different clinical trials utilizing the same study medication.

## 7 MEASUREMENT OF CLINICAL AND IMMUNOLOGICAL EFFICACY

7.1 Methods of Malignant Disease Evaluation - Each patient will have a baseline evaluation prior to the injection of the Ad/PSA vaccine. The measurements will include temperature, weight, serum PSA, blood chemistries, a quantitative bone scan for bone metastases, and CT for soft tissue metastases, and performance status for quality of life.

Patients will be seen in the GCRC (see Table 5 for schedule). The injection site will be examined for evidence of erythema, induration and necrosis and patients will have their temperature and weight recorded and interviewed to determine whether they experienced any adverse reactions. Blood samples will also be taken for measurement of PSA and anti-PSA antibodies (see Table 5). At the 6 month, 12 month, and subsequent semi-annual visits each patient will be evaluated using the measurements listed for the baseline visit.

7.2 Scans – Bone scans will be performed every three months for the first year and every 6 months thereafter. If there is measurable disease by CT scan at registration then CT scans will also be performed using the same schedule as the bone scans.

7.3 Use of Serum PSA for Disease Evaluation – Based upon our pre-clinical experiments and the results from the Phase I clinical trial we expect the immunized men to produce anti-PSA antibodies. The levels of antibody will be measured by a flow cytometry assay as described by Cavacini, et al. used in our Phase I clinical trial (40). We will also explore the use of a second serum marker for prostate cancer, hK2 in collaboration with Donald Tindall, Charles Young, and George Clee at the Mayo Clinic. Investigators at Mayo, along with Hybritech, Inc. have been exploring hK2 and published a number of papers in recent years on the subject (67-70). Patient sera from each clinic visit will be sent to Mayo where they will measure the levels of hK2. We will use the data to evaluate the effect of anti-PSA antibodies on both PSA and hK2 in the sera of vaccinated patients.

### 7.4 Experimental Evaluation of the Ad/PSA Vaccination

7.4.1 Blood will be collected prior to, and at each visit after, the injection of the Ad/PSA vaccine. Two separate samples will be collected; one in red top tubes to allow collection of serum from coagulated blood and a second in heparinized tubes to permit collection of lymphocytes.

7.4.2 Levels of PSA, hK2, anti-PSA antibodies, and anti-adenovirus antibodies will be measured in the serum.

- 7.4.3 Anti-PSA T cell immune responses will be measured by ELISPOT analysis using the methods developed for, and used in, our Phase I clinical trial. In addition to measuring the anti-PSA T cell activity, we will also measure anti-adenovirus T cell activity as well as reactivity to stimulation with cytomegalovirus (CMV). A non-specific stimulus will be provided by PMA and ionomycin for each patient's lymphocytes.

## 7.5 Definitions of Response –

### 7.5.1 Primary Endpoint - PSA doubling-time response

- 7.5.1.1 Definition: a 50% increase in the PSADT compared to pre-enrollment PSADT.
- 7.5.1.2 PSADT will be calculated based on the MSKCC calculator, available at <http://www.mskcc.org/mskcc/html/10088.cfm>.
- 7.5.1.3 PSADT response will be measured at 9 and 18 months after initiation of study treatment.
- 7.5.1.4 Three measurements of PSA, spaced at least 2 weeks apart, will be required prior to study enrollment. Post-treatment PSADT will be based on PSA levels at 3, 6 and 9 months (9 month PSADT calculation) and 3,6,9,12,15,18 month levels (18 month PSADT calculation).

### 7.5.2 Secondary Endpoint – PSA response

- 7.5.2.1 Definition: a 50% reduction in the pre-treatment PSA value, verified with a second measurement 30 days later.

### 7.5.3 Progression:

- 7.5.3.1 In men with previously negative bone scan at entry - positive bone scan
- 7.5.3.2 In men with previously positive bone scan at entry - doubling of PSA and/or changes in bone scan.
- 7.5.3.3 In men with measurable disease by CT at entry – progressive change

### 7.5.4 Onset of Response – The time between initiation of therapy and the onset of PR or CR.

### 7.5.5 Duration of Response – Time from onset of PR or CR, whichever occurs first, (even if the patient later has a CR) until objective evidence of progression.

## 8 STUDY PARAMETERS

8.1 Scans or x-rays used to document measurable or evaluable disease should be done with 4 weeks prior to study entry

8.2 CBC with differential, LFT's should be done  $\leq 2$  weeks before study entry. Castrate levels of testosterone

8.3 All chemistries should be done  $\leq 2$  weeks before the study entry, unless specifically required on day 1 as per protocol. If abnormal, they must be repeated within 48 hours prior to study entry.

8.4 Hgb, Hct, WBC, Plt should be done  $\leq 2$  weeks before study entry but, if abnormal, they must be repeated <48 hours prior to study entry.

8.5 REMOVAL OF PATIENTS FROM STUDY (Criteria for discontinuation of a patient's study participation)

8.5.1 Adverse events: In the event of a vaccine-associated unmanageable or irreversible toxicity, the investigator will withdraw a patient from further treatment and notify the Study Chair immediately. *In addition, the FDA and the IRB will be notified of the adverse events.*

8.5.2 Disease Progression: Patients will be taken off-study if they have progressive disease (PD) or clinically significant deterioration at any time during the study if the investigator feels that (a) alternative prostate cancer therapy might benefit the patient, or (b) to continue on study might be unsafe for the patient. Patients receiving alternative prostate cancer therapies will still be followed for toxicity and immunologic evaluations.

8.5.3 Allergic Reactions: Patients will be removed from the study should they develop grade II allergic reactions.

8.5.4 Personal Reasons: As stated in the informed consent, patients may withdraw from the study at any time.

8.5.5 Clinical Judgment: A patient may be withdrawn from the study, if, in the opinion of the investigators, it is not in the patient's best interest to continue (e.g. an adverse experience, intercurrent illness, etc.)

8.5.6 The date of discontinuation and the reason(s) for patient discontinuation from the study will be recorded in the CRF. All evaluations that are required at the follow-up must be conducted for each patient who discontinues treatment, regardless of the reason.

### Regulatory and Reporting Requirements

The Data and Safety Monitoring Committee (DSMC) of the Holden Comprehensive Cancer Center will provide data and safety monitoring for this study. "The Data and Safety Monitoring Plan of the Holden Comprehensive Cancer Center" provides standard operating procedures to monitor all clinical cancer trials at the UIHC. All investigator-initiated trials are automatically



monitored by the DSMC. A detailed data and safety monitoring plan for this study is on file with the DSMC and the Clinical Research Safety Officer (CRSO).

### **Data Management, Quality Control and Data Security**

In order to protect confidentiality the subject will be assigned an identification number. This number will be used on all specimens from the subject and will be used for documentation purposes.

Data management for the optimal entry, processing, storage, and retrieval for this protocol's data will be accomplished by the principal investigator. The database will be located on a computer or in a locked cabinet in a locked office. This computer will be secured, accessible only by the research team. There will be more than one copy of the database. The second, secured, copy of the protocol data will be stored in a locked room accessible only by the research team. For quality control, auditing, and checking data for integrity, there will be a regular accounting of data periodically performed

**Table 5**  
**Study Design and Testing**

	Prior to Study Entry	At 1 <sup>st</sup> Ad/PSA injection	During the first 24 hours	30 d.	44 d.	60 d.	74 d.	90 d.	6 mo	9 mo	12 mo	Every 6 mo. to prog.
Physical Examination	X	X	x	X	X	X	X	X	X	X	X	X
Performance Status	X			X	X	X	X	X	X	X	X	X
Vital Signs	X	X	x	X	X	X	X	X	X	X	X	X
Weight	X	X		X	X	X	X	X	X	X	X	X
Blood for PSA, PAP	X	X		X	X	X	X	X	X	X	X	X
Blood for anti-PSA Ab, and lymphocytes for cellular immunity	X	X		X	X	X	X	X	X	X	X	X
Hgb/Hct, WBC, platelets	X	X	x	X	X	X	X	X	X	X	X	X
Differential	X		x	X	X	X	X	X	X	X	X	X
AST, ALT, LDH, alkaline phosphatase, bilirubin	X		x	X	X	X	X	X	X	X	X	X
Creatinine	X		x	X	X	X	X	X	X	X	X	X
Urinalysis	X		x	X	X	X	X	X	X	X	X	X
Chest x-ray	X			X		X		X	X	X	X	X
Bone scan	X							X <sup>3</sup>	X		X	X
Abdominal/pelvic CT	X								X		X	X
EKG	X								X		X	X

<sup>3</sup> If measurable disease by CT at registration.

## 9 DRUG FORMULATION AND PROCUREMENT

### 9.1 Drug Name

Adenovirus/PSA (Ad/PSA)

### 9.2 Classification

Vaccine

### 9.3 Mode of Action

The adenovirus is a replication-deficient virus unable to produce virus progeny in the infected cells. The virus will infect cells in the location of the injection site, the PSA gene will produce the protein product which will be recognized as an antigen by the immune system and produce anti-PSA immune responses. Based upon our pre-clinical studies in an animal model of human prostate cancer, these responses, mainly the CD8+ CTL response, will cause the destruction of PSA-secreting prostate tumors.

### 9.4 Dose Specifics and Route of Administration

The route of injection, vehicles for the vaccine, and dose schedules have been outlined in Section 6.1 of this protocol.

### 9.5 Availability

Produced and provided by Molecular Medicine, LLC, San Diego, CA

### 9.6 Manufacturing

- 9.6.1 The PSA cDNA provided by Donald Tindall, Mayo Clinic, Rochester, MN, was placed 3' to the CMV promoter in a shuttle vector containing Ad5 DNA. The sequence inserted was the pre-pro form of PSA described by Lundwall (71) that encodes 262 amino acids with a predicted molecular weight of 28.8 kDa. Using methods previously described (72), the shuttle vector and E1a-E1b deletion mutant Ad5 DNA were transfected into HEK 293 cells, and recombination between the DNA species was allowed to occur. The amplification and purification of Ad/PSA was performed by the University of Iowa Gene Transfer Vector Core as previously described (73). Ad/lacZ used as a control was also obtained from the Gene Transfer Vector Core and is previously described (74).
- 9.6.2 The Principal Investigator provided the Ad/PSA vaccine used for the pre-clinical studies to Molecular Medicine, LLC of San Diego, CA for the production of the clinical grade product. Information on the manufacturing of the GMP Vaccine by Molecular Medicine, LLC is found in the accompanying documents supplied by the company.

## 10 STATISTICAL CONSIDERATIONS

- 10.1 **Statistics** - The ideal endpoint would be a clinical outcome that is of particular relevance to the patient such as increased time to tumor progression, increased time to death or reducing the proportion of death. This trial is using a surrogate endpoint as a substitute to the clinically meaningful outcome since the tumor cannot be accessed directly. The association between the surrogate and survival rate had not been clearly established by any phase I & II trial. The trial consists of using Ad/PSA vaccine administered in multiple injections to prostate cancer patients with hormone—refractory metastatic prostate cancer—with the goal to induce anti-PSA T cells responses. Three injections of equal dose are proposed. The previous phase I trial consisting of a single injection in men with hormone-refractory metastatic prostate cancer was able to induce anti-PSA T cells responses. The Phase I consisting of a single injection using dose escalation protocol of the vaccine in an aqueous or matrix delivery vehicle did not show any significant AE. Additional pre-clinical pharmacology /toxicology studies required by the FDA did not show any significant side effects using the three-injection schedule. The primary endpoint is the serum PSADT.

For efficacy purposes, we expect at least 50% of the patients to show 50% increase in PSADT. This proportion is judged clinically important and anything less than 30% can stop the trial for futility. The ideal design would be a two-stage design of Simon (74) requiring 32 patients. After testing the treatment on 12 patients, if 3 did not have a 50% increase in PSADT, the trial will be terminated. Otherwise, an additional 20 patients will be recruited for the trial. The expected sample size will be 19.73 and the probability of early termination, 0.72. If the treatment is not effective, there is 0.1 probability of concluding that it is. If the treatment is effective, there will be 0.2 probability of concluding that it is not. However, due to the complexity of the trial, we will not use Simon (74) but a more conservative approach consisting of using 31 patients in a one-stage design at efficacy level and a two-stage design at toxicity level for stopping rules. By using a one-stage design at efficacy endpoint, we are making the probability of early termination for efficacy purposes to be zero. Early termination will be based on toxicity only. The reason for this design is due to the nature of the treatment and is explained below. Enrollment will not stop unless stopping rules based on toxicity are satisfied:

*“After the third vaccine is administered, toxicity will be evaluated 90 days into the trial”*

Due to the nature of the treatment, anti-tumor activity will be potentially delayed and the primary efficacy endpoint will be determined 18 to 20 months after initial patient accrual. Since we will be able to assess PSADT after nearly 20 months into the study, it will be unreasonable to stop the study due to unsatisfactory results prior to that point. This is the main reason why we will carry a one-stage design for the primary endpoint and a two-stage design for toxicity. Criteria for proceeding with enrollment into a subsequent stage prior to the two-year efficacy evaluation will be based on assessment of toxicity in the patient cohorts since this can be done as early as 90 days into the trial.

The optimal two-stage design to test the null hypothesis that the toxicity level is less than 15% versus the alternative that it is greater than 35% has an expected sample

size of 19.51, and a probability of early termination of 0.57. If the toxicity level is high, there will be a 0.043 probability of concluding that it is not. If the level is actually low, there is a 0.19 probability of concluding that it is not. After enrolling 11 patients in the first stage, the trial will be terminated if 4 or higher show grade 3 toxicity or higher. After testing the vaccine on 31 patients in the first and second stages, the trial will be terminated if 7 or more show grade 3 toxicity or higher. This corresponds to testing the hypothesis that  $p_0 > 35\%$  versus  $p_1 < 15\%$  sequentially and in three stages at a fixed significance level of 0.05. The reasons for using a two-stage design instead of one-stage are because we are using a surrogate endpoint that has not been formerly proved to have an ease of predictability of an outcome of direct relevance to patients; also, it is not quite clear how these surrogates relate to the pathway of the natural disease and to overall survival rate.

## 10.2 Reference Sources for Statistical Considerations

*Simon, Richard. "Optimal Two-Stage Designs for Phase II Clinical Trials," Controlled Clinical Trials, 1989, Volume 10, pages 1-10.*

*De Gruttola Victor et Al., "Considerations in the Evaluation of Surrogate Endpoints in Clinical Trials: Summary of NIH workshop" Controlled Clinical Trials, 2001, Volume 22, pages 485-502*

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Lubaroff, David M.

## **INFORMED CONSENT DOCUMENT – PROTOCOL #1**

**Project Title: Phase II study of adenovirus/PSA vaccine in men with recurrent prostate cancer after local therapy**

**Research Team:** David M. Lubaroff, PhD; Richard D. Williams, MD; Fadi Joudi, MD; Daniel Vaena, MD; Mark C. Smith, MD, Tammy Madsen PA; Pamela Zehr, BSN, MSN; Carlene Etscheidt, BSN, MSN; Gideon Zamba, PhD

### **WHAT IS THE PURPOSE OF THIS STUDY?**

This is a research study. We are inviting you to participate in this research study because you have recurrent cancer of the prostate. This investigational study involves treatment with an Ad/PSA vaccine. This is a virus vaccine in which the gene for prostate specific antigen (PSA) has been placed into a common cold virus termed adenovirus (Ad) to produce this Ad/PSA product. The adenovirus used in this clinical trial will not be infectious and therefore, will not cause any cold symptoms. PSA is produced by normal and cancerous prostate cells. Since you may have previously been treated for your prostate cancer by surgery or irradiation the only cells that will be secreting PSA are the remaining cancer cells and therefore have a therapeutic effect on your cancer.

We have chosen you because you have a small amount of cancer as indicated by your recent evidence of recurrent disease. We plan to vaccinate you three times, each injection administered at 30 day intervals. Based upon our earlier clinical trial the vaccine is safe and should not induce any major side effects. Importantly, this treatment should not cause any major side effects as would treatment with anti-cancer drugs.

### **HOW MANY PEOPLE WILL PARTICIPATE?**

Approximately 46 people in each part of this protocol will take part in this study at the University of Iowa and the Iowa City Veterans Affairs Medical Center

### **HOW LONG WILL I BE IN THIS STUDY?**

If you agree to take part in this study, your involvement will last for approximately 2 years, perhaps longer. Much depends upon the effect of the vaccination on your cancer.

### **WHAT WILL HAPPEN DURING THIS STUDY?**

You will first be evaluated by members of the Department of Urology and/or Internal Medicine and Radiation Oncology to determine whether you meet the eligibility criteria for entrance into the study. As part of that determination you will be subject to a physical examination, x-rays and scans, an electrocardiogram, and blood will be taken for laboratory tests. This will take place in a clinic at the University of Iowa Hospitals and Clinics or the Iowa City VA Medical Center. Once your eligibility has been confirmed you will be entered into the study. You will be injected with the vaccine in the thigh

and kept overnight in the General Clinical Research Center to be certain that you do not develop any side effects, although none are expected. You will return to the clinic (Urology, Internal Medicine, or VA) 30 days after the first vaccination where more blood will be taken to repeat the laboratory tests and you then receive a second vaccination. Return visits will be scheduled 14 and 30 days after the second vaccination. More laboratory tests will be performed on your blood and a third and final vaccination will be administered at visit 30 days after the second vaccination; this will be 60 days after the initial vaccination. Return visits for additional testing will occur 74 days after the first vaccination (14 days after the third), 90 days, 60 months, 12 months, and every 6 months thereafter until the end of the study. At the 6 month intervals you will also have additional x-rays, scans, and electrocardiograms.

## **WHAT ARE THE RISKS OF THIS STUDY?**

There may be some risks from being in this study. This study uses a form of gene transfer. Since gene transfer with the Ad/PSA vaccine is a new clinical trial for the treatment of prostate cancer not all of the risks associated with this study treatment are known. In a previous trial using adenoviruses, a death did occur. However, results from our Phase I trial of the vaccine indicated that none of the patients suffered any vaccine-related serious side effects. Based upon our Phase I trial, the adenovirus vaccine may cause a local inflammation at the site of injection, a decrease in white blood cell count (which could result in an increased risk of infection), headaches, or fever. The types and frequency of side effects observed in the Phase I trial include localized reddening or swelling at the injection site (28%), flu or cold-like symptoms (16%), groin or injection site pain (9%), fatigue with protein in the urine (9%), decreased count of a specific type of white blood cells (3%). All side effects were transient and either resolved by themselves or treated with aspirin or other mild pain relievers.

There may be some local tenderness, reddening, or swelling at the injection site, but this will normally disappear within a few days. The PSA vaccine may induce antibodies against PSA protein. This could interfere with the ability of your doctor to monitor your PSA blood levels. The reason is that the antibodies to PSA may lower the amount of the PSA protein in your blood that would not reflect a clinical change in your cancer. We are working with other scientists to study this possibility. Although the vaccine may lower your serum PSA levels it will not interfere with your doctor's ability to follow your disease. We are not certain how long the potential for lowering your serum PSA levels will last during the study. We will carefully monitor both your PSA levels and measurable cancer.

Your physician will be checking you closely to see if any side effects are occurring. Routine blood and urine tests will be done to monitor the effects of treatment. Many side effects disappear after the drug is stopped. In the meantime, your doctor may prescribe medications to keep these side effects under control.

## **Are there any Unforeseen Risks?**

We do not expect any unforeseen risks, although we cannot be 100 percent certain since this is a new therapy. We rely on the results of our Phase I clinical trial that indicated that the vaccine is a safe product..

## **WHAT ARE THE BENEFITS OF THIS STUDY?**

We don't know if you will benefit from being in this study. The primary goal of the study is to determine whether the vaccination with the adenovirus/PSA vaccine will benefit patients by eliminating PSA-secreting prostate tumor cells. However, we hope that, in the future, other people might benefit from this study as data obtained from this Phase II clinical trial may indicate the level of benefit obtained from the vaccination.

## **WHAT OTHER TREATMENT OPTIONS ARE THERE?**

Alternatives which could be considered in your case include hormonal therapy if you are not already on this treatment. Your study doctor can provide detailed information about your disease and the benefits of hormonal therapy. You should feel free to discuss your disease and prognosis with your doctor.

Before you decide whether or not to be in this study, the study physician involved in your care will be available to answer any questions you have concerning this program. In addition, you are free to ask your study physician any questions concerning this program that you wish in the future. You will be advised of the procedures related solely to research that would not otherwise be necessary.

## **REQUEST FOR AUTOPSY**

To obtain vital information about the safety and efficacy of gene transfer at the time of a death, no matter what the cause, permission for an autopsy will be requested of from your family. Please advise your family of the request and of its scientific and medical importance.

## **WILL IT COST ME ANYTHING TO BE IN THIS STUDY?**

No compensation for participation will be given. The vaccine Ad/PSA and the costs of administration will be provided to you without cost. The expense of the medical care involved will be borne either by you or third party payers.

## **WILL I BE PAID FOR PARTICIPATING?**

You will not be paid for being in this research study.

## **WHO IS FUNDING THIS STUDY?**

Research grants are funding this research study. This means that the University of Iowa is receiving payments to support the activities that are required to conduct the study. No one on the research team will receive a direct payment or increase in salary for conducting this study.

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- If you are injured or become ill from taking part in this study, medical treatment is available at the University of Iowa Hospitals and Clinics.

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Lubaroff, David M.

- No compensation for treatment of research-related illness or injury is available from the University of Iowa unless it is proven to be the direct result of negligence by a University employee.
- If you experience a research-related illness or injury, you and/or your medical or hospital insurance carrier will be responsible for the cost of treatment.

## **WHAT ABOUT CONFIDENTIALITY?**

We will keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people may become aware of your participation in this study. For example, federal government regulatory agencies, and the University of Iowa Institutional Review Board (a committee that reviews and approves research studies) may inspect and copy records pertaining to this research. Some of these records could contain information that personally identifies you.

In the future, the granting agency may continue to use your health information that is collected as part of this study. For example, they may combine information from this study with the results of other studies to re-analyze the safety and effectiveness of the study medication, to evaluate other products or therapies, to develop a better understanding of a disease, or to improve the design of future research studies. The funding agency may also share information from this study with regulatory agencies in foreign countries.

To help protect your confidentiality, we will assign a specific coded number to your file that will appear on the data forms and files, all written files will be kept in a locked office and information on computers will be protected by secure passwords. If we write a report or article about this study or share the study data set with others, we will do so in such a way that you cannot be directly identified.

The Informed Consent Document will be placed in your medical record.

## **WILL MY HEALTH INFORMATION BE USED DURING THIS STUDY?**

The Federal Health Insurance Portability and Accountability Act (HIPAA) requires the University of Iowa Health Care to obtain your permission for the research team to access or create “protected health information” about you for purposes of this research study. Protected health information is information that personally identifies you and relates to your past, present, or future physical or mental health condition or care. We will access or create health information about you, as described in this document, for purposes of this research study and for your treatment. Once the University of Iowa Health Care has disclosed your protected health information to us, it may no longer be protected by the Federal HIPAA privacy regulations, but we will continue to protect your confidentiality as described under “Confidentiality.”

We may share your health information related to this study with other parties including federal government regulatory agencies, the University of Iowa Institutional Review Boards and support staff,

You cannot participate in this study unless you permit us to use your protected health information. If you choose *not* to allow us to use your protected health information, we will discuss any non-research alternatives available to you. Your decision will not affect your right to medical care that is not research-related. Your signature on this Consent Document authorizes the University of Iowa Health

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Lubaroff, David M.

Care to give us permission to use or create health information about you.

Although you may not be allowed to see study information until after this study is over, you may be given access to your health care records by contacting your health care provider. Your permission for us to access or create protected health information about you for purposes of this study has no expiration date. You may withdraw your permission for us to use your health information for this research study by sending a written notice to David M. Lubaroff, PhD, Department of Urology, 200 Hawkins Drive, Iowa City, IA 52242. However, we may still use your health information that was collected before withdrawing your permission. Also, if we have sent your health information to a third party, such as the study sponsor, or we have removed your identifying information, it may not be possible to prevent its future use. You will receive a copy of this signed document.

### **Interest of the Media and Others in the Research**

Others may have an interest in the innovative character of the protocol and in the status of the treated subjects. The University of Iowa and the Iowa City VA Medical Center and the investigators will make efforts to provide protection from the media in an effort to protect all participants' privacy. Also, representatives of applicable Federal agencies (e.g., the [National Institutes of Health](#) and the [Food and Drug Administration](#)) will have access to the subjects' medical records.

### **IS BEING IN THIS STUDY VOLUNTARY?**

Taking part in this research study is completely voluntary. You may choose not to take part at all. If you decide to be in this study, you may stop participating at any time. If you decide not to be in this study, or if you stop participating at any time, you won't be penalized or lose any benefits for which you otherwise qualify.

### **What if I Decide to Drop Out of the Study?**

If you withdraw from the study, you will continue to be monitored and clinical data will continue to be collected from your medical records. You will be given a copy of this consent form for your records.

### **Will I Receive New Information About the Study while Participating?**

If we obtain any new information during this study that might affect your willingness to continue participating in the study, we'll promptly provide you with that information.

### **Can Someone Else End my Participation in this Study?**

Under certain circumstances, the researchers or the study sponsor might decide to end your participation in this research study earlier than planned. This might happen for any one or more of the following reasons: (a) in our judgment it would not be safe for you to continue, (b) because your condition has become worse, (c) because funding for the research study has ended, (d) the data and safety monitoring committee has closed the trial due to a low number of patients entered into the trial..

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## WHAT IF I HAVE QUESTIONS?

We encourage you to ask questions. If you have any questions about the research study itself, please contact: Richard D. Williams, MD at (319) 356-0760 or Dr. Fadi Joudi at (319) 384-5993 of the Department of Urology, or Dr. Daniel Vaena (319) 356-1616 of the Department of Internal Medicine Hematology/Oncology. If you are calling after hours please call 319-356-1616 and ask for the Urology Resident or Oncology Fellow on call.

If you have questions about the rights of research subjects or research related injury, please contact the Human Subjects Office, 300 College of Medicine Administration Building, The University of Iowa, Iowa City, Iowa, 52242, (319) 335-6564, or e-mail [irb@uiowa.edu](mailto:irb@uiowa.edu). General information about being a research subject can be found by clicking "Info for Public" on the Human Subjects Office web site, <http://research.uiowa.edu/hso>.

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This Informed Consent Document is not a contract. It is a written explanation of what will happen during the study if you decide to participate. You are not waiving any legal rights by signing this Informed Consent Document. Your signature indicates that this research study has been explained to you, that your questions have been answered, and that you agree to take part in this study. You will receive a copy of this form.

Subject's Name (printed): \_\_\_\_\_

\_\_\_\_\_  
(Signature of Subject)

\_\_\_\_\_  
(Date)

## Statement of Person Who Obtained Consent

I have discussed the above points with the subject or, where appropriate, with the subject's legally authorized representative. It is my opinion that the subject understands the risks, benefits, and procedures involved with participation in this research study.

\_\_\_\_\_  
(Signature of Person who Obtained Consent)

\_\_\_\_\_  
(Date)

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Lubaroff, David M.

## **INFORMED CONSENT DOCUMENT – PROTOCOL #2**

**Project Title:** Phase II study of Adenovirus/PSA vaccine in men with hormone - refractory prostate cancer

**Research Team:** David M. Lubaroff, PhD; Richard D. Williams, MD; Fadi Joudi, MD; Daniel Vaena, MD; Mark C. Smith, MD, Tammy Madsen PA; Pamela Zehr, BSN, MSN; Carlene Etscheidt, BSN, MSN; Gideon Zamba, PhD

### **WHAT IS THE PURPOSE OF THIS STUDY?**

This is a research study. We are inviting you to participate in this research study because you have recurrent cancer of the prostate. This investigational study involves treatment with an Ad/PSA vaccine. This is a virus vaccine in which the gene for prostate specific antigen (PSA) has been placed into a common cold virus termed adenovirus (Ad) to produce this Ad/PSA product. The adenovirus used in this clinical trial will not be infectious and therefore, will not cause any cold symptoms. PSA is produced by normal and cancerous prostate cells. Since you may have previously been treated for your prostate cancer by surgery or irradiation the only cells that will be secreting PSA are the remaining cancer cells. The purposes of this study are to determine whether immunization with the Ad/PSA vaccine will induce an anti-PSA immunity that will result in the destruction of the remaining prostate cancer cells and therefore have a therapeutic effect on your cancer..

We have chosen you because you have hormone refractory metastatic prostate cancer. We plan to vaccinate you three times, each injection administered at 30 day intervals. Based upon our earlier clinical trial the vaccine is safe and should not induce any major side effects. Importantly, this treatment should not cause any major side effects as would treatment with anti-cancer drugs.

### **HOW MANY PEOPLE WILL PARTICIPATE?**

Approximately 32 people will take part in this study at the University of Iowa and the Iowa City Veterans Affairs Medical Center..

### **HOW LONG WILL I BE IN THIS STUDY?**

If you agree to take part in this study, your involvement will last for approximately 2 years, perhaps longer. Much depends upon the effect of the vaccination on your cancer.

### **WHAT WILL HAPPEN DURING THIS STUDY?**

You will first be evaluated by members of the Department of Urology and/or Internal Medicine and Radiation Oncology to determine whether you meet the eligibility criteria for entrance into the study. As part of that determination you will be subject to a physical examination, x-rays and scans, an electrocardiogram, and blood will be taken for laboratory tests. This will take place in a clinic at the University of Iowa Hospitals and Clinics or the Iowa City VA Medical Center. Once your eligibility has been confirmed you will be entered into the study. You will be injected with the vaccine in the thigh



and kept overnight in the General Clinical Research Center to be certain that you do not develop any side effects, although none are expected. You will return to the clinic (Urology, Internal Medicine, or VA) 30 days after the first vaccination where more blood will be taken to repeat the laboratory tests and you then receive a second vaccination. Return visits will be scheduled 14 and 30 days after the second vaccination. More laboratory tests will be performed on your blood and a third and final vaccination will be administered at visit 30 days after the second vaccination; this will be 60 days after the initial vaccination. Return visits for additional testing will occur 74 days after the first vaccination (14 days after the third), 90 days, 60 months, 12 months, and every 6 months thereafter until the end of the study. At the 6 month intervals you will also have additional x-rays, scans, and electrocardiograms.

### **WHAT ARE THE RISKS OF THIS STUDY?**

There may be some risks from being in this study. This study uses a form of gene transfer. Since gene transfer with the Ad/PSA vaccine is a new clinical trial for the treatment of prostate cancer not all of the risks associated with this study treatment are known. In a previous trial using adenoviruses, a death did occur. However, results from our Phase I trial of the vaccine indicated that none of the patients suffered any vaccine-related serious side effects. Based upon our Phase I trial, the adenovirus vaccine may cause a local inflammation at the site of injection, a decrease in white blood cell count (which could result in an increased risk of infection), headaches, or fever. The types and frequency of side effects observed in the Phase I trial include localized reddening or swelling at the injection site (28%), flu or cold-like symptoms (16%), groin or injection site pain (9%), fatigue with protein in the urine (9%), decreased count of a specific type of white blood cells (3%). All side effects were transient and either resolved by themselves or treated with aspirin or other mild pain relievers.

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### **Are there any Unforeseen Risks?**

We do not expect any unforeseen risks, although we cannot be 100 certain since this is a new therapy. We rely on the results of our Phase I clinical trial that indicated that the vaccine is a safe product..

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Lubaroff, David M.

## **WHAT ARE THE BENEFITS OF THIS STUDY?**

We don't know if you will benefit from being in this study. The primary goal of the study is to determine whether the vaccination with the adenovirus/PSA vaccine will benefit patients by eliminating PSA-secreting prostate tumor cells. However, we hope that, in the future, other people might benefit from this study as data obtained from this Phase II clinical trial may indicate the level of benefit obtained from the vaccination.

## **WHAT OTHER TREATMENT OPTIONS ARE THERE?**

Alternatives which could be considered in your case include hormonal therapy if you are not already on this treatment. Your study doctor can provide detailed information about your disease and the benefits of hormonal therapy. You should feel free to discuss your disease and prognosis with your doctor.

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## **REQUEST FOR AUTOPSY**

To obtain vital information about the safety and efficacy of gene transfer at the time of a death, no matter what the cause, permission for an autopsy will be requested of from your family. Please advise your family of the request and of its scientific and medical importance.

## **WILL IT COST ME ANYTHING TO BE IN THIS STUDY?**

No compensation for participation will be given. The vaccine Ad/PSA and the costs of administration will be provided to you without cost. The expense of the medical care involved will be borne either by you or third party payers.

## **WILL I BE PAID FOR PARTICIPATING?**

You will not be paid for being in this research study.

## **WHO IS FUNDING THIS STUDY?**

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## **WHAT IF I AM INJURED AS A RESULT OF THIS STUDY?**

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Lubaroff, David M.

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## **WHAT ABOUT CONFIDENTIALITY?**

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Lubaroff, David M.

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### **Interest of the Media and Others in the Research**

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If you withdraw from the study, you will continue to be monitored and clinical data will continue to be collected from your medical records. You will be given a copy of this consent form for your records.

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If you have questions about the rights of research subjects or research related injury, please contact the Human Subjects Office, 300 College of Medicine Administration Building, The University of Iowa, Iowa City, Iowa, 52242, (319) 335-6564, or e-mail [irb@uiowa.edu](mailto:irb@uiowa.edu). General information about being a research subject can be found by clicking "Info for Public" on the Human Subjects Office web site, <http://research.uiowa.edu/hso>.

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This Informed Consent Document is not a contract. It is a written explanation of what will happen during the study if you decide to participate. You are not waiving any legal rights by signing this Informed Consent Document. Your signature indicates that this research study has been explained to you, that your questions have been answered, and that you agree to take part in this study. You will receive a copy of this form.

Subject's Name (printed):

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(Signature of Subject)

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(Date)

### Statement of Person Who Obtained Consent

I have discussed the above points with the subject or, where appropriate, with the subject's legally authorized representative. It is my opinion that the subject understands the risks, benefits, and procedures involved with participation in this research study.

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(Signature of Person who Obtained Consent )

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(Date)

## **INVESTIGATOR'S BROCHURE**

### **ADENOVIRUS/PSA VACCINE**

**Edition No. 2**

**Release Date: May 18, 2006**

THE INFORMATION IN THIS INVESTIGATIONAL DRUG BROCHURE IS CONFIDENTIAL. Disclosure of the information contained in this document is permitted only as necessary to obtain informed consent from patients receiving this study drug or their legal guardians, or for disclosure with persons involved in study conduct, ethical or institutional review, or regulatory authorities. All other disclosure requires written permission from the sponsor, University of Iowa.

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## **I. Prostate Cancer**

Prostate cancer has emerged as a major public health concern. The lifetime risk for developing cancer of the prostate (CaP) in American males is one in five. Although there is no known cause for CaP, there are several factors that may increase the risk of CaP development, including genetics, race, age, or diet (1-3). It has been reported that certain chromosomal regions contain risk factors for CaP, and consistent with this, an individual's risk more than doubles if a close relative has CaP, with two relatives, it increases fivefold, and with three relatives, risk is virtually 100%. Blacks are twice as likely to be diagnosed with CaP and have twice the mortality rate than that of whites. Asian men have the lowest incidence of CaP, but upon emigration to the US, their rates rise to almost that of whites (4). Genetics, testosterone levels, and diet are believed to play a part in these racial differences. In addition, risk of CaP increases with age. Men over 65 years of age are at the highest risk; however, 25% of all reported cases are diagnosed under the age of 65.

CaP incidence rates increased 141.8% between 1973 and 1994, and in 1998, new cases totaled over 180,000. In 2006, it is estimated that 27,350 men will die from CaP in the United States (5). This cancer continues to be the most frequently diagnosed malignancy, aside from skin cancers, representing 29% of all new cancer cases in US men, and the mortality rate is second only to heart disease in this group.

According to the National Cancer Institute, as measured by lost wages, productivity, and medical costs, CaP costs up to \$15 billion annually, and currently, the federal government spends 50 times more in patient care than in research to find a cure.

## **II. Currently Available Treatments**

CaP can be a difficult disease to detect and treat. It is a multi-focal disease, i.e. there is often more than one focus of malignant cells in the organ, and often varying stages of differentiation exist between individual foci. Treatment options are limited to surgery or radiation therapy for localized disease. Surgical treatment (prostatectomy) is most common among younger, healthier patients in whom gross metastatic events have been ruled out; however, this treatment can have side effects that severely compromise the patient's quality of life such as incontinence and sexual dysfunction. Radiation therapy is less invasive and involves either the directing of x-rays into the pelvic area, or implanting radioactive pellets into the prostate. However, all forms of radiotherapy are associated with complications, including acute cystitis, prostatitis, enteritis, and urinary/sexual dysfunction.

In patients with metastatic CaP, androgen ablation is palliative therapy that serves to reduce tumor burden and maximize patient longevity. This is achieved by medical or surgical castration. However, hormonal therapy can have significant side effects. Not all patients can tolerate the drugs, and almost all lose sexual function. Several hormonal therapies exist to eliminate androgens. Surgical removal of the testis will reduce testosterone levels to 5-10%, and when combined with bilateral adrenalectomy or treatment with aminoglutethimide, testosterone levels become undetectable. Administration of diethylstilbestrol, an estrogen, has been useful, although it is associated with severe cardiovascular side effects. Currently in use are the luteinizing hormones releasing hormone (LHRH) agonists. These are powerful stimulators of the hypothalamus, causing it to release luteinizing hormone (LH), which stimulates the production of testosterone. In the presence of LHRH agonists, the body fails to make normal LHRH, there is no release of LH, and serum levels of testosterone falls to castrate levels. To further inhibit the action of androgen, non-steroidal antiandrogens are used in



conjunction with LHRH agonists. The mechanism of non-steroidal antiandrogens is not completely understood, but they block dihydrotestosterone, the active form of testosterone, from stimulating protein synthesis in prostate cells. Although these forms of hormonal therapy will eliminate hormone-sensitive cells and reduce tumor burden by approximately 80%, the remaining hormone-resistant disease will continue to proliferate and eventually result in the death of the patient. No effective treatment for hormone-refractory prostate cancer is available. Because of prostate cancer's obvious medical ramifications, there is a great need for the development of an effective treatment.

### III. Immunotherapy

In the early twentieth century Coley used bacterial infections to initiate an antitumor response (6). Although not understood, these observations formed the basis for the supposition that immuno-adjuvant therapy could override tumor escape mechanisms and induce an antitumor response. The general promise of this hypothesis failed to materialize into clinically effective therapy, although adjuvant BCG therapy for bladder cancer emerged as an effective treatment regimen (7). The overall lack of success of these adjuvant immunotherapy regimens lead to doubt about the ability of the immune response to effectively eliminate tumors.

Rosenberg and associates revitalized interest in immunotherapy with their work on LAK and TIL (8-10). These experiments demonstrated the presence of immune cells that could be activated *in vitro*. The *in vitro* activated cells mediated antitumor activity on adoptive transfer into tumor-bearing hosts (8). Again, the therapeutic efficacy of clinical trials fell short of expectations. However, the studies clearly demonstrated the ability of immune cells to eliminate tumors previously considered to be resistant to immune effector mechanisms (10).

Gene therapy studies confirmed the hypothesis that most theoretically "non-immunogenic" tumors were indeed immunogenic (11-18). These studies demonstrated that expression of cytokines or co-stimulatory molecules in sufficient quantities at the tumor site induced an antitumor response. Neither the systemic administration of cytokines nor the production of cytokines by transfected cells at sites distant from the tumor induced an antitumor response (14-18). Only rarely did cytokine gene therapy induce regression of existing tumors at secondary sites, and this occurred only in the early growth stages (12). In some systems regression of small tumor burdens could be induced by multiple immunizations with IL2-transfected tumor cells (18).

The use of microbial vectors to carry foreign proteins as vaccines in cancer therapy has been documented in a number of experimental systems (reviewed in 19). A few examples of this research include Rosenberg's group use of replication-defective adenovirus vaccines to elicit anti-tumor immunity in mouse models of colon carcinoma and melanoma (20-22). Herlyn and colleagues have effectively demonstrated the use of adenoviral vaccines in the treatment of a mouse colon carcinoma (23). From these data and those of others, it is clear that recombinant microbial vaccines appear to lead to more effective antigen presentation in the tumor cell. This may be a direct result of tumor antigen synthesis within the antigen-presenting cell (APC) or may be a consequence of antigen expression outside the suppressive effects of the tumor (19).

Several possible target antigens for immunotherapy that are unique to the prostate have been identified. These include PSA (hK3), human glandular kallikrein II (hK2), prostate-specific membrane antigen (PSMA), and prostatic acid phosphatase. These antigens are produced by normal prostate epithelial cells and most prostate cancer cells, whether androgen-dependent or androgen-independent. Our laboratory as well as the laboratories of other investigators have

demonstrated the presence of both antibody production and T cell reactivity to PSA (24-31 & unpublished observations). Since these are normal antigens that activate T cell responses *in vitro*, it is probable that the absence of an immune response to the antigens is associated with the expression of peripheral tolerance in the form of anergy. Thus, studies have been proposed that would develop viral and bacterial vectors expressing these antigens in order to abrogate the anergic state and induce antitumor immunity.

The development of immunotherapy protocols for the treatment of human prostate cancer using PSA is dependent on the ability to overcome immune tolerance to the antigen. Studies in numerous autoimmune and tolerance models demonstrate that tolerance mechanisms can be abrogated and that the resulting immune response is tissue destructive. The hypothesis that forms the foundation for research used as the basis for this clinical trial is that activation of the immune response to prostate-associated antigens will initiate an antitumor response.

#### IV. Background on the Vaccine

- a. Adenovirus Vaccines - Recombinant adenoviral vectors transduce a wide range of dividing and nondividing cells types, making this gene delivery system valuable as a tool for studying diseases, for vaccine therapy, and for potential clinical use (32). Recombinant adenovirus can be prepared and purified in high titers. In addition, wild-type adenovirus infections are extremely common in the general population, giving adenovirus a well-documented safety record (33). Moreover, adenovirus are structurally stable and no side effects have been reported following the vaccination of US military recruits with wild types, demonstrating their safety for human use (34). Adenoviral vectors for gene therapy and vaccine therapy are adenoviruses that have been genetically modified to allow insertion of foreign genes and to render the virus replication-defective. Current vectors have a deletion in the E1 region or in both the E1 and E2 regions. Adenoviral gene transfer has been used in a variety of experimental conditions that include transfers to the liver (35), lung (36), central nervous system (37,38), and to cancer cells (39).

There is evidence that the introduction of foreign transgenes into hosts by adenovirus induces a CTL response to the transgene product that is ultimately responsible for the elimination of the virus (32,40). While this is disadvantageous in situations where gene therapy is being used to insert functional genes into host cells, it is advantageous in the use of viruses carrying foreign genes as immunogens. In the vaccine therapy of cancer, active immunization against a murine colon cancer antigen and melanoma antigens have been induced by adenoviral vaccines (41,42).

- b. Adenovirus/PSA Vaccine - The PSA gene was placed 3' to the CMV promoter in a shuttle vector containing Ad5 DNA. The sequence inserted was the pre-pro form of PSA described by Lundwall (43). The gene encodes for 262 amino acids with a predicted molecular weight of 28.8 kDa. Using methods previously described (44), the shuttle vector and E1a-E1b deletion mutant Ad5 DNA were transfected into HEK 293 cells, and recombination between the DNA species was allowed to occur. The amplification and purification of Ad/PSA was performed by the University of Iowa Gene Transfer Vector Core as previously described (45). The recombinant adenovirus/PSA vaccine will be referred to as Ad/PSA.
- c. Gelfoam<sup>®</sup> Matrix - Gelfoam (Pharmacia & Upjohn Company, Kalamazoo, MI) is a medical device intended for application to bleeding surfaces as a hemostatic agent. It is

a water-insoluble, off-white, non-elastic, porous, pliable product prepared from purified pork skin. The Gelfoam gelatin preparation is available either as a cross-linked sponge or as non-cross linked beads. It is able to absorb and hold within its interstices approximately 45 times its weight of blood and other fluids (46). The absorptive capacity of Gelfoam is a function of its physical size, increasing with increasing gelatin volume (47).

## V. Pre-Clinical Information

Pre-clinical experiments were performed in mice using the Ad/PSA for the treatment of prostate cancer. The hypothesis for these studies is that immunization with an adenovirus vaccine, carrying the gene for human PSA, would induce anti-PSA immune responses. These responses would consist of both antibody and immune T cells, both cytotoxic T lymphocytes (CTL) and T-helper (TH), as measured by *in vitro* assays. This anti-PSA immunity should result in the ability of the immune animals to destroy PSA-producing tumor *in vivo*.

To date the results of our studies include:

1. A replication-defective adenovirus type 5 expressing human PSA was produced (48, attached publication). This will be referred to as Ad/PSA. Control virus was adenovirus type 5 transformed with the lacZ gene.
2. For the animal studies the RM11 mouse prostate tumor model, generated by Thompson from his mouse prostate reconstitution model was chosen. Although this mouse prostate tumor does not secrete PSA, the human PSA gene was transfected into the cells driven by a CMV promoter. Stable expression of the protein was demonstrated and the PSA-transfected prostate tumor line has been referred to as RM11/PSA. Cloned cell lines from the RM11/PSA cultures were isolated and the ability to produce tumors in recipient mice and to serve as target cells in our CTL assays has been demonstrated.
3. In initial experiments, Balb/c mice were immunized with  $10^9$  plaque forming units (pfu) of the Ad/PSA vaccine or the control Ad/lacZ vaccine by both the subcutaneous (sc) and intraperitoneal (ip) routes. Differences in the immune responses generated by using the two routes of immunization will be indicated throughout this summary. Two weeks after immunization the mice were bled, killed and their spleens removed. Cell suspensions were prepared and tested for anti-PSA cell-mediated immunity in proliferation and cytotoxicity assays. For the CTL responses the population of PSA-specific T cells were expanded by a 5-day culture of the spleen cells and mitomycin C-treated stimulator cells. These latter cells were the syngeneic P815 tumor cells that were previously transfected with the same human PSA gene. The serum from the blood was tested for the presence of anti-PSA antibodies by ELISA. The data from these experiments demonstrated the presence of both humoral and cell-mediated immunity to PSA. The following have been documented: a). high concentrations of anti-PSA antibody, b). strong proliferative response following stimulation with 10  $\mu$ g of purified PSA, and c). vigorous CTL responses using clone E5 of the RM11/PSA tumors as target cells. Controls of mice immunized with Ad/lacZ did not demonstrate any anti-PSA immunity nor was there any cell killing of non-PSA secreting RM11/neo target cells.
4. Different doses of the Ad/PSA vaccine were compared for their ability to induce anti-PSA immunity, mainly using the CTL response as a measure. Using the ip route of immunization doses of  $10^9$  pfu and  $10^8$  pfu gave identical responses, while immunization

with  $10^7$  pfu induced a modest response;  $10^6$  pfu and  $10^5$  pfu did not induce any anti-PSA responses (Figure 1). A dose of  $10^9$  pfu given sc also induced the production of strong humoral and cellular anti-PSA responses, but the strength of the induced responses declined thereafter. The dose of  $10^8$  pfu induced a modest response, similar to the response generated by  $10^7$  pfu administered ip. Doses less than  $10^7$  pfu were incapable of inducing a response following this single immunization schedule. *In all future experiments the dose of vaccine will be  $10^9$  pfu, unless stated otherwise.*

5. The next set of experiments was designed to determine how long the anti-PSA CTL responses were maintained. Thus, mice were immunized at varying times and the CTL assays run on the same day so that CTL activity was analyzed at 1, 2, 3, 4, 5, 11, and 26 weeks after immunization. The data indicated that the strongest responses were detected at 2 and 3 weeks, with the data indistinguishable at the two time points (Figure 2). Lesser, but still strong responses were detected at all other times. They were virtually identical at all times up to the 26-week observation.
6. Evidence that the PSA-specific lysis is mediated by CD8+ CTL was obtained from experiments in which specific T cell populations were depleted or blocked. For these studies, 100  $\mu$ g of 2.43 anti-CD8 monoclonal antibody and GK1.5 anti-CD4 monoclonal antibody were injected ip. into mice for three consecutive days before splenic harvest. The anti-NK antibody anti-asialo GM1 (asGM1) was injected once at 25  $\mu$ g/mouse three days before splenic harvest. The control antibody used was a non-specific rat IgG2b. All injection volumes were 100  $\mu$ l. Splenocytes isolated from Ad/PSA-immunized mice depleted of CD8+ T cells did not lyse PSA-expressing targets (Figure 3). Furthermore, the addition of 2.43 antibody in lytic assays blocked the ability of splenocytes from normal Ad/PSA-immunized mice to lyse PSA-expressing targets. *In vivo* depletion of CD4+ T cells or NK cells did not abrogate this lysis. Thus, as expected, CD8+ T cells mediated all of the in vitro CTL activity against PSA.
7. Experiments to determine whether the strong CTL responses induced by a primary immunization with Ad/PSA will protect mice *in vivo* from a challenge with the mouse RM11/PSA tumor have been completed. One of the cloned tumor lines that are uniform in their expression of the protein was used in these experiments. Mice were immunized either with Ad/PSA or Ad/lacZ and 2 weeks later injected either with clone 6 of RM11/PSA or the control, RM11/*neo* cells. The results of these experiments indicated that all of the control mice produced large tumors and were killed at three weeks. The University of Iowa Animal Care Unit guidelines require the euthanasia of animals with tumors equal or greater than 20% of their body size. Small tumors were produced in some of the mice immunized with Ad/PSA and challenged with the PSA-producing E6 tumors in the first three weeks after injection (Figure 4). In 70% of the mice the tumors regressed. Although the true survival of the mice cannot be measured when taking the point at which we must euthanize mice due to large, life-threatening tumors, none of the control mice survived, while 70% of the immunized mice survived (Figure 5). These data are very exciting because the experiments demonstrate, in this model system, that the generation of anti-PSA immunity affords protection against the growth of PSA-producing prostate tumors *in vivo*.
8. The PSA-specific responses induced by Ad/PSA showed the generation of reactive CD4+ and CD8+ T cells and antibody responses. To determine which effector arm is responsible for mediating the protective effects against PSA-expressing prostate tumor growth, mice were immunized ip. with  $1 \times 10^9$  pfu Ad/PSA or Ad/lacZ. On days 11, 12,

and 13 after immunization, groups of Ad/PSA-immunized mice were injected with the control antibody SFR8-B6, the CD8+, T cell-depleting antibody 2.43, or the CD4+ T cell-depleting antibody GK1.5. The NK-depleting antibody anti-asialo GM1 was injected on day 11 and every four days thereafter. At day 14, all mice were challenged sc. with  $1 \times 10^5$  RM11/PSA tumor cells. The data indicated that in Ad/PSA-immunized mice, depletion of CD8+ T cells abrogated the protective effects of Ad/PSA (Figure 6). One hundred per-cent of mice in the CD8-depleted group developed tumors. However, tumor size in CD8-depleted mice was significantly smaller than tumors in control mice, suggesting that other effector components contribute to the antitumor effects of Ad/PSA. Consistent with this observation, depletion of CD4+ T cells resulted in an increase in tumor outgrowth, indicating that this T cell population also contributes to the inhibition of RM11/PSA growth in Ad/PSA-immunized mice. NK cells do not appear to participate in the anti-tumor activity.

9. Although these data indicated that immunization with the Ad/PSA vaccine induced strong anti-PSA responses and these responses could afford mice a protective immunity against PSA-secreting prostate tumors, it was important to compare the Ad/PSA vaccine to other PSA viral vectors. A replication competent vaccinia that had been transformed with the human PSA gene was obtained from Dr. Jeffrey Schlom of the National Cancer Institute. This vaccine, derived from the Wyeth vaccine strain of vaccinia (Prostvac), was compared to Ad/PSA, a replication deficient vaccinia (NYVAC/PSA), and a canarypox (ALVAC/PSA) for their ability to induce anti-PSA CTL. All of the transformed viruses contained the same number of peptides associated with the H-2<sup>d</sup> haplotype. Balb/c mice were immunized with the Prostvac, Ad/PSA, NYVAC/PSA, or ALVAC/PSA viruses and control viruses that did not contain the PSA gene. *All doses were at  $10^8$  pfu since this was the highest dose that could be used with the poxvirus vaccine..* In the three experiments, adenovirus and Prostvac were clearly superior to both NYVAC/PSA and ALVAC/PSA. The data also indicated that Ad/PSA was more effective than Prostvac (Figure 7).
10. To more accurately compare the anti-PSA immunity induced by the different PSA vaccines, two-color flow cytometric analysis was used to quantitate the production of antigen-specific CD8+/interferon- $\gamma$  (IFN $\gamma$ )+ cells. This procedure allows accurate measurement of the effector cell frequency. The anti-PSA response was measured using this method at days 7, 10, and 14 after immunization to insure that the peak response was the same to all vaccines. Data from these experiments indicated that the anti-PSA responses induced by Ad/PSA, Prostvac, and NYVAC/PSA peaked 14 days after immunization. When comparing the effector cell frequency of T cells from these immunized mice, Ad/PSA was again shown to be superior to Prostvac and NYVAC/PSA, and ALVAC/PSA (Table 1).
11. In order to test the biological significance of this PSA vaccine hierarchy, mice immunized with the different vaccines and analyzed for their ability to protect mice from challenge with the RM11/PSA tumors. Mice were immunized as described for the *in vitro* experiments above and injected with  $1 \times 10^5$  RM11/PSA clone E6 prostate tumor cells two weeks later. Because of the absence of significant anti-PSA immunity induced by ALVAC/PSA we did not include this vaccine the *in vivo* experiments. As with the *in vitro* CTL data, the Ad/PSA vaccine was superior to all other viruses. The hierarchy in these studies was Ad/PSA > vaccinia/PSA > NYVA/PSA (Figure 8 and 9).

12. In collaboration with Dr. Timothy Ratliff and Dr. D. Robert Siemens, the induction of strong anti-PSA responses was examined by comparing sc immunization with the virus in an aqueous suspension to absorption of the virus suspension to a Gelfoam<sup>®</sup> matrix. In these experiments mice were immunized with  $10^7$ ,  $10^8$ , or  $10^9$  pfu either as an aqueous sc injection as before, or in the gelfoam matrix. Two weeks later some mice were killed and a CTL assay performed on their splenocytes and the remaining mice were challenged with clone E6 of the RM11/PSA prostate tumor cells. The results from these studies indicated that immunization with Ad/PSA in matrix was superior to immunization with the aqueous vaccine (Figure 10 and 11). This was reflected both in the CTL activity and protection of mice from tumor challenge.
13. One of the challenges that we will face in a clinical trial is the effect that anti-adenovirus antibodies may have on the ability of an adenoviral vaccine to induce immune responses to transgene products. The majority of the patient population has existing antibodies to adenovirus type 5 as the result of normal contact with this agent. In preclinical studies preimmunization of mice with the Ad/lacZ virus was shown to induce the production of high levels of anti-adenovirus antibody. These mice have a reduced CTL response to subsequent immunization with the Ad/PSA vaccine. Immunization of Ad/lacZ injected mice with Ad/PSA in matrix produced strong anti-PSA CTL responses, equal to the responses in mice not preimmunized to adenovirus. Apparently the virus when imbedded in a solid matrix is able escape the immediate effects of the antibodies and allows the generation of anti-PSA responses to the transgene product (49, attached publication).
14. Mice were weighed following injection of Ad/PSA vaccine in doses ranging from  $10^6$  through  $10^9$  pfu. No apparent toxicity was demonstrated as all mice gained weight equal to that of mice not injected with the vaccine.
15. In preparation for both the Phase I and Phase II trials, we carried out a series of Pharmacology/Toxicology studies. In both series no adverse events were noted in any of the mice, in the histopathology of 11 organs, in the hematology and clinical chemistry values. This was true whether a single injection was given in the pre-Phase I series, with or without the collagen matrix, or the three injections given in the pre-Phase II series.

## VI. Phase I study results

The protocol for the Phase I trial was published and the paper is attached to this document (50). The median age of the patients was 70.2 years (range, 52 to 89). The vaccine was administered as an aqueous suspension or in a collagen (Gelfoam) matrix to 32 patients. Sixteen (16/32) or 50% of the patients exhibited grade 1 vaccine-related adverse events (AE), 1/32 (3.1%) that exhibited a grade 2 AE, and one patient exhibited a grade 3 AE which was a decrease in neutrophil count. There were no vaccine-related grades 4 or 5 AEs. Table 2 demonstrates the adverse events, listed by body system.

We measured the anti-PSA immune responses, both antibody and T cell, in all patients enrolled in the study. Antibody responses to PSA were measured by the binding to PSA-secreting cell lines using the method adapted from Cavacini, et al. (51). Results of those analyses demonstrated that 57% of men immunized with the Ad/PSA vaccine developed measurable anti-PSA antibodies. ELISPOT assays were utilized to measure anti-PSA T cell responses. The results, depicted in Table 3, demonstrate that of the 32 patients, 18 (56.3%) developed anti-

PSA T cell responses. The addition of Gelfoam did not appear to affect the development of anti-PSA responses, but in this Phase I study the numbers of patients in each group was too small to make statements of statistical significance of the data. These results demonstrate the ability of men with late stage metastatic prostate cancer, injected one time with Ad/PSA, to respond to the vaccine with the production of anti-PSA T cells.

The effects of vaccination on serum PSA and on patient survival were also evaluated as a secondary endpoint in the phase I trial. Although there was no sustained decline in individual PSA levels, the PSA doubling times (PSADT, calculated based on 3 pre-enrollment consecutive PSA measurements) were reduced in 54 percent of the patients compared to pre-vaccine administration, with the best responses occurring in patients immunized with the highest dose of the vaccine (Table 4). In addition, published survival nomograms for patients with hormone refractory prostate cancer were applied to patients in this phase I trial (52,53). Table 5 shows that 57% of all patients at all doses, whether injected with the vaccine as an aqueous suspension or in the collagen matrix, had a survival time longer than that predicted by the nomogram. The range of increased survivals in the different groups was 33% to 100%.

## **VII. Recommendations for Clinical Use**

Based on the significant pre-clinical activity of the Ad/PSA vaccine in generating tumor-specific T cells, and the encouraging safety and efficacy results from our phase I study, we propose to continue the clinical development of the Ad/PSA vaccine with the performance of the current phase II trial. It is our contention that the vaccine product and the method of immunization set this therapy apart from other ongoing prostate cancer investigational immunotherapeutic approaches. Specifically, the incorporation of Gelfoam, not present in other vaccine preparations, enhances the induction of strong anti-PSA responses. Immunization of mice with Ad/PSA in Gelfoam matrix was able to induce anti-PSA responses even in the presence of high-titer anti-adenovirus antibodies. Notably, most humans naturally possess high titers of anti-adenovirus antibodies due to natural exposure to adenoviruses.

We plan to enroll two populations of prostate cancer patients into the Phase II clinical trial. The ideal patient population to determine a therapeutic benefit of a new treatment, particularly immunotherapy, is one with minimal disease burden. The low tumor burden should allow therapies, particularly those relying on antigen-specific effector T lymphocytes, to destroy all of the cancerous tissues and cells. The first population will be men with recent evidence of recurrence following surgery or radiation therapy for their primary tumor. Patients in the population will be eligible if they exhibit at least four separate rises in serum PSA, at least one month apart with differences  $\geq 0.03$  ng/ml and a total PSA of  $>0.2$  ng/ml; have a PSA doubling time of  $\geq 6$  months; not at high risk as determined by the Kattan nomogram (54) or patients that refuse treatment with Taxotere or radiation. All patients will be hormone naïve. Since standard therapy for these patients would be to postpone androgen ablation therapy until such time as there is a high serum PSA level ( $\geq 20$  ng/ml), enrolling patients into this Phase II trial does not withhold accepted treatment. Patients will be excluded from the trial if they are candidates for salvage radiation therapy, had multiple positive margins at surgery, had a serum PSA of  $>20$  ng/ml prior to surgery, a Gleason score of  $>7$ , seminal vesicle involvement or positive lymph nodes.

The second population to be enrolled in the trial will be men with recurrent disease who are undergoing androgen depletion therapy. The choice of this additional patient population is based upon published documentation that inflammation and the generation of immune responses are augmented by hormone withdrawal (55-57). Mercader, et al., in attempts to

demonstrate an enhanced termination of tolerance to prostate associated antigens documented CD4+ and CD8+ T cell infiltrates in benign prostates and in prostate tumors of men undergoing androgen withdrawal (55). Roden and co-workers published data demonstrating that T cell levels and T cell proliferation were increased in mice following castration (56) while Drake, et al. reported breaking tolerance to antigens associated with the TRAMP prostate tumors in mice (57). Therefore, we propose to vaccinate men beginning 14 days after the initiation of androgen withdrawal using the same three injection protocol.

### VIII. Packaging, Storage, and Handling

The vaccine, Manufactured by Molecular Medicine, LLC or San Diego, CA under Good Manufacturing Practices (GMP), was packaged as follows: The virus was suspended in buffer (100 mM NaCl, 25 mM Tris buffer, 10mg/ml lactose) and filtered through a 0.2 micron filter. Dilutions of the vaccine to obtain the proper number of pfu used the same buffer. The material was then placed in sterile 1.5 ml cryovials, with each vial containing 250 microliters.

The vaccine will be stored in a freezer at  $-80^{\circ}\text{C}$  housed in a limited access facility. Only individuals actively involved in the clinical trial will have access to the freezer. For each patient, one vial, containing the precise number of pfu, will be removed from the freezer and thawed at room temperature. Care will be taken not to agitate the contents, with mixing to be carried out by gentle swirling of the fluid in the vials. No dilutions of the contents will be necessary, as the vaccine has been packaged as one dose per vial. One hundred microliters of the vaccine will be removed from the thawed vials, mixed with Gelfoam<sup>®</sup>, and administered subcutaneously to the patient. The used vial will be discarded in a biological waste container.

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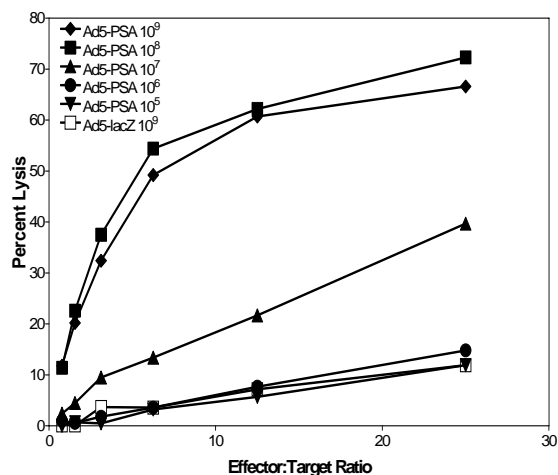
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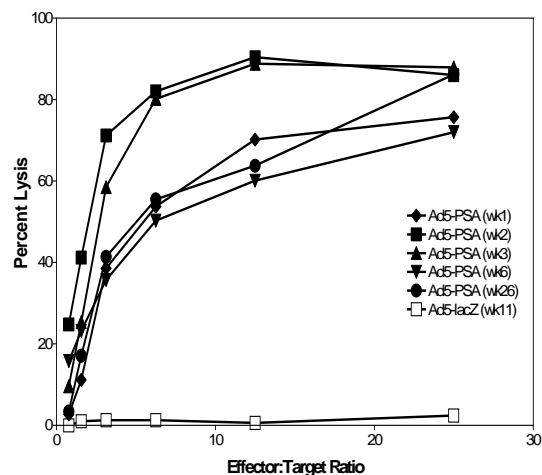
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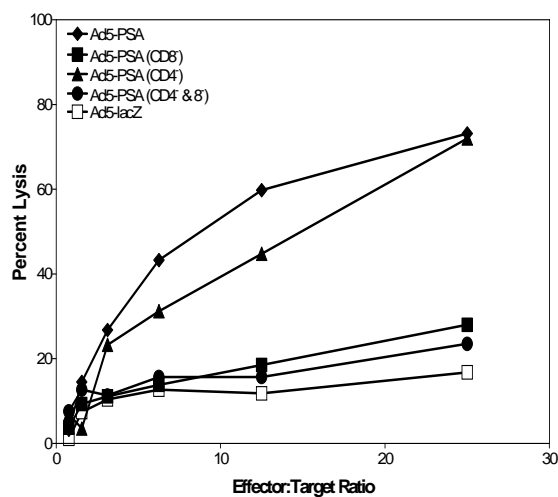
**Figure 1**

Mice were immunized with varying doses of Ad/PSA vaccine and spleen cells tested for CTL activity 14 days later. Target cells were RM11/PSA. Control targets of RM11/neo were not lysed (data not shown).



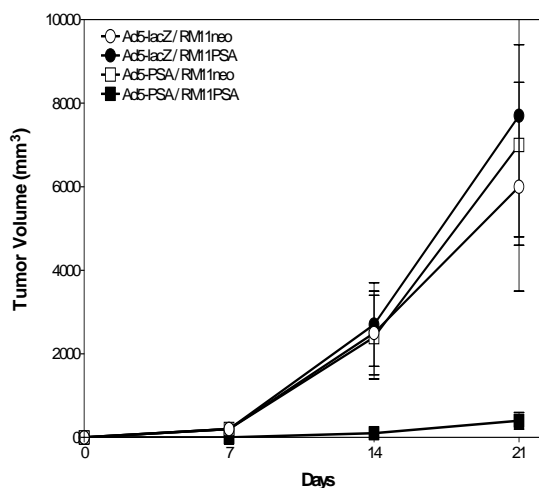
**Figure 2**

Mice were immunized with  $10^9$  pfu of the Ad/PSA vaccine and the spleen cells tested against RM11/PSA target cells at varying times afterwards. Control targets of RM11/neo were not lysed (data not shown).



**Figure 3**

Mice were immunized with the Ad/PSA vaccine. Fourteen days later they were injected with antibody to CD4 and/or CD8 T cells. Their cells were then tested for anti-PSA CTL against RM11/PSA targets (shown) and control RM11/neo targets (not shown).



**Figure 4**

Mice were immunized as before and injected with either RM11/PSA or control RM11/neo tumor cells. Tumors were measured weekly and volumes calculated.

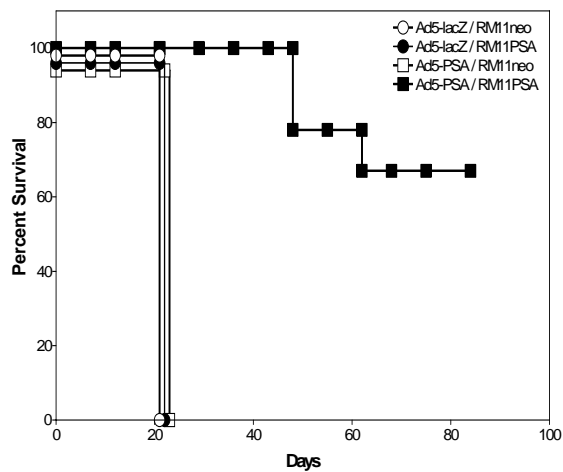


Figure 5

Mice were immunized as before and injected with either RM11/PSA or control RM11/neo tumor cells. Mice were killed when tumors exceeded 20% of body weight. Long-term survivors were free of tumor.

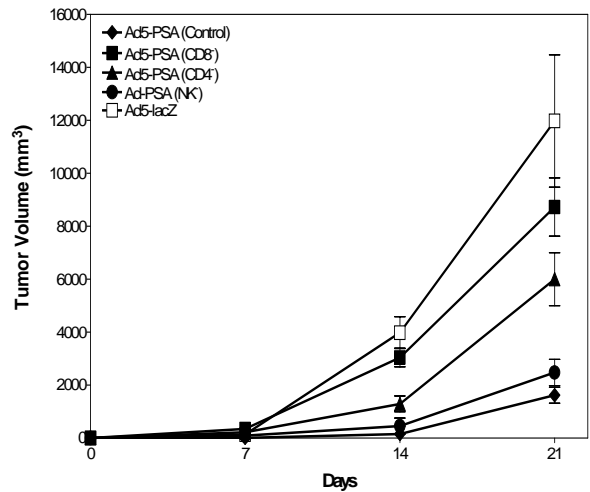


Figure 6

Mice were immunized with the Ad/PSA vaccine. Fourteen days later they were injected with antibody to CD4 and/or CD4 T cells. The mice were then injected with either RM11/PSA or control RM11/neo tumor cells. Tumors were measured weekly and volumes calculated.

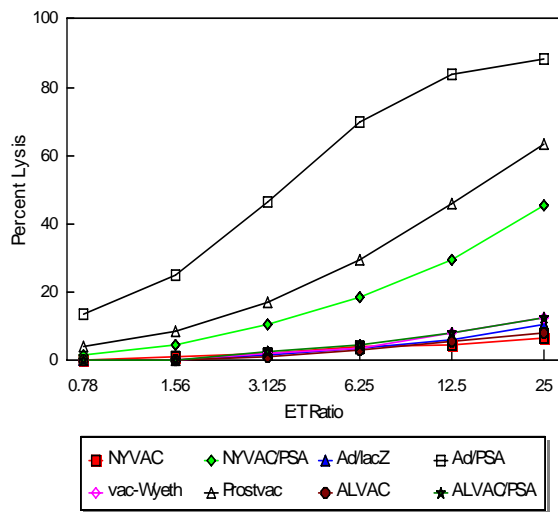


Figure 7

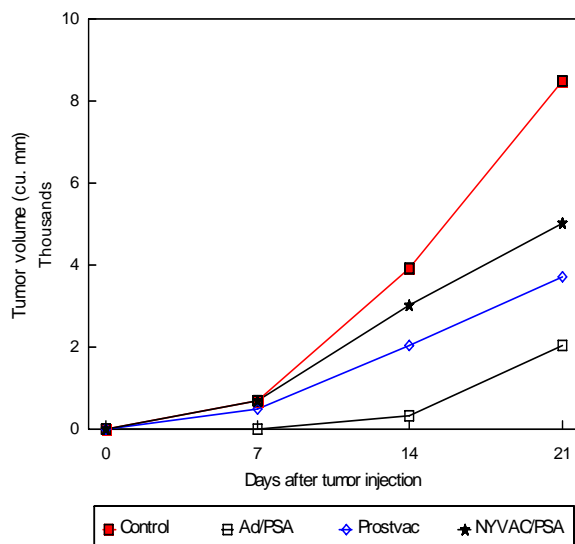
Mice were immunized with the various PSA-carrying vaccine and 2 weeks later CTL assays performed using spleen cells. Data represent lysis of RM11/PSA target cells. Control RM11/neo target cells were not lysed (not shown)

### Effector Frequency Analysis

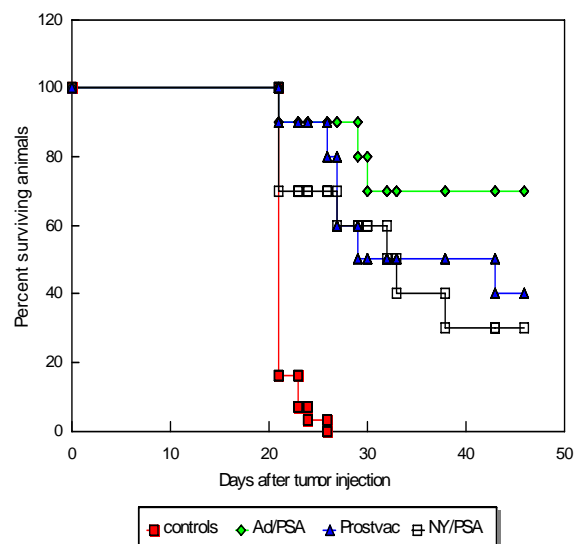
Vaccine	Number of PSA-Specific CD8+ Cells per 10 <sup>6</sup> CD3+ T Cells
Ad/PSA	2196
Prostvac	493
NYVAC/PSA	278
ALVAC/PSA	28

Table 1

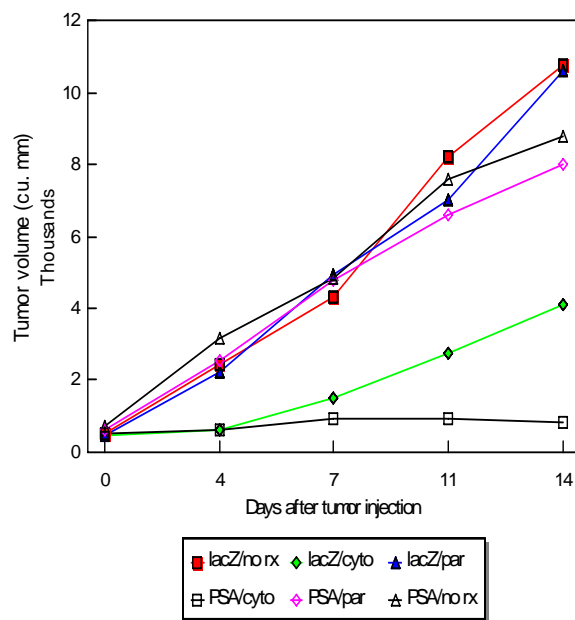
Number of anti-PSA effector cells in the spleen of mice immunized with equal doses of the different vaccines. Effector cells were enumerated by analyzing the number of CD8+IFN $\gamma$ + cells in the spleen following a 4 hour stimulation for A20/PSA cells. Values have been corrected by subtracting background values from unstimulated cells.

**Figure 8**

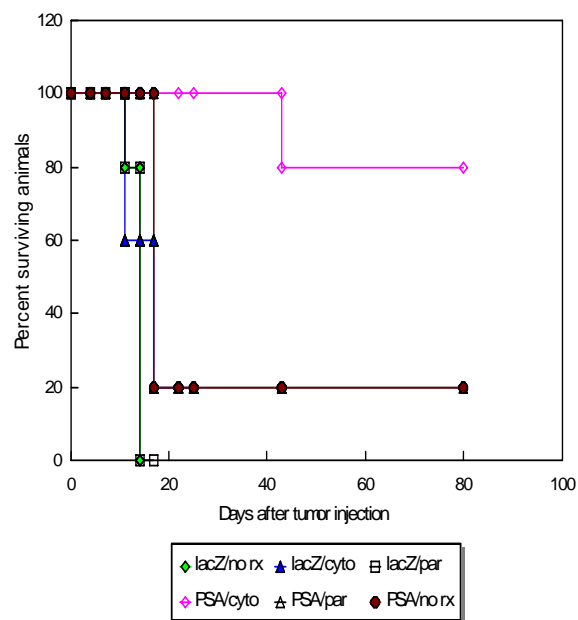
Tumor protection of mice immunized with the different PSA carrying viruses. Mice were immunized with equal doses of the vaccines and injected with either RM11/PSA or control RM11/neo tumor cells. The data in the figure represents the growth of RM11/PSA tumors. The RM11/neo injected mice all produced large tumors (not shown).

**Figure 9**

Survival of mice immunized with the different PSA carrying viruses. Mice were immunized with equal doses of the vaccines and injected with either RM11/PSA or control RM11/neo tumor cells. The data in the figure represents the survival of mice injected with RM11/PSA tumors. None of the mice injected with the RM11/neo survived greater than 21 days (not shown).

**Figure 10**

Destruction of established tumors in mice injected with Ad/PSA and/or ALVAC/cytokine viruses. Mice were injected with either RM11/PSA or RM11/neo tumor cells and immunized with either Ad/PSA or Ad/lacZ viruses 3 days later. Seven days after immunization some groups of mice received an intratumoral injection of the ALVAC/ cytokine virus. Data represent the growth of the RM11/PSA tumors. None of the control RM11/neo

**Figure 11**

Survival of mice injected with Ad/PSA and/or ALVAC/cytokine viruses. Mice were injected with either RM11/PSA or RM11/neo tumor cells and immunized with either Ad/PSA or Ad/lacZ viruses 3 days later. Seven days after immunization some groups of mice received an intratumoral injection of the ALVAC/ cytokine virus. Data represent the survival of mice injected with the RM11/PSA tumors. None of the control RM11/neo tumors survived past 21 days(not shown).

**Table 2**  
**Ad/PSA Phase I Trial**  
**Adverse Events – by System**

<b>Patient</b>	<b>System</b>	<b>Day of onset*</b>	<b>Event</b>	<b>Vaccine-related</b>
AP012	Cardiovascular	21	Hypotension	Unlikely
AP015	Cardiovascular	137	Myocardial infarction	Unlikely
AP019	Cardiovascular	1	Hypotension	Possible
AP026	Cardiovascular	14	Lower extremity edema	No
AP034	Cardiovascular	14	Hypotension/dizziness	Unlikely
AP036	Cardiovascular	63	Edema – bilateral ankles	Unlikely
AP006	Constitutional	14	Chills	Unlikely
AP014	Constitutional	22	Fatigue	No
AP019	Constitutional	1	fever	Possible
AP019	Constitutional	14	fatigue	Possible
AP035	Constitutional	64	Weight loss	No
AP009	GI	21	Constipation	No
AP012	GI	0	Heartburn	Unlikely
AP020	GI	21	increased alkaline phosphatase	Possible
AP022	GI	2	constipation	No
AP025	GI	85	abdominal distention, constipation, apparently related to metastatic disease in the periaortic lymph nodes and periprostatic tumor mass	Unlikely
AP027	GI	1	GI cramping	No
AP027	GI	1	constipation	No
AP035	GI	50	Constipation	No
AP037	GI	73	Nausea	No
AP008	GU	21	Proteinuria	Possibly
AP010	GU	21	Proteinuria	No
AP016	GU	172	Left nephrolithiasis	No
AP016	GU	183	New primary tumor – papillary bladder	No
AP019	GU	1	Proteinuria	Possible
AP020	GU	21	ketonuria	Possible
AP035	GU	22	Proteinuria	No
AP010	Hematologic	1	Decrease in absolute neutrophils count	Possibly
AP014	Hematologic	82	Anemia	Unlikely
AP021	Hematologic	1	decreased lymphocytes	Unlikely
AP021	Hematologic	63	thrombocytopenia	Unlikely
AP022	Hematologic	14	decreased lymphocyte count	
AP022	Hematologic	63	decreased WBC & neutrophils	No
AP024	Hematologic	14	anemia	Unlikely
AP024	Hematologic	1	anemia	Unlikely
AP025	Hematologic	14	thrombocytopenia	Unlikely
AP035	Hematologic	15	Lymphopenia	No
AP036	Hematologic	1	Anemia	Possibly
AP036	Hematologic	63	Anemia	Possibly
AP037	Hematologic	1	Lymphopenia	Possibly
AP016	Immune	8	Flu-like symptoms	No
AP023	Immune	16	urinary tract infection	Unlikely
AP027	Immune	21	Infection	Unlikely
AP023	Liver	21	elevated AST	Unlikely
AP027	Liver	21	increased AST	Possibly

<b>Table 2 - continued</b> <b>Ad/PSA Phase I Trial</b> <b>Adverse Events – by System</b>				
AP020	Metabolic	14	hyponatremia	Possible
AP020	Metabolic	14	hyperglycemia	Possible
AP002	Musculoskeletal	3	Groin pain	Unlikely
AP005	Musculoskeletal	1	Left inguinal pain	Possibly
AP006	Musculoskeletal	13	Left hip and thigh pain	No
AP007	Musculoskeletal	22	Left back pain	No
AP009	Musculoskeletal	14	Joint aches, secondary to fall	No
AP014	Musculoskeletal	14	Leg pain (bone – femur)	No
AP016	Musculoskeletal	4	Bone pain – left leg	No
AP016	Musculoskeletal	21	Decreased left leg strength	No
AP018	Musculoskeletal	8	Bilateral rib pain	No
AP022	Musculoskeletal	5	increased bone pain	
AP023	Musculoskeletal	20	pain in left buttocks	No
AP025	Musculoskeletal	16	bone pain	No
AP034	Musculoskeletal	14	Back pain	Unlikely
AP035	Musculoskeletal	22	Increased alkaline phosphatase	No
AP002	Neural	3	headache	Unlikely
AP005	Neural	21	Agitation	No
AP006	Neural	14	Migraine headache	Unlikely
AP007	Neural	14	Situational depression	No
AP009	Neural	11	Fall, causing head lacerations & loss of consciousness	No
AP012	Neural	21	Dizziness	Unlikely
AP018	Neural	8	Insomnia	No
AP020	Neural	168	cord compression	No
AP022	Neural	16	depression	
AP025	Neural	122	Headache	Unlikely
AP003	Pulmonary	21	Pleural effusion	Unlikely
AP008	Respiratory	3	Cold symptoms	Possibly
AP015	Respiratory	1	Cough	No
AP018	Respiratory	43	DOE?	Unlikely
AP020	Respiratory	9	viral symptoms	Possible
AP034	Respiratory	prior	Edema, wheezing (change in inhaler in same time period)	No
AP002	Skin	1	Itching	Unlikely
AP002	Skin	0	hematoma at injection site	Yes
AP003	Skin	0	Bruising at injection site	Yes
AP004	Skin	0	Ecchymosis at injection site	Yes
AP005	Skin	0	Ecchymosis and erythema at injection site	Yes
AP008	Skin	0	Ecchymosis at injection site	Yes
AP009	Skin	0	Ecchymosis at injection site	Yes
AP010	Skin	21	Edema	No
AP018	Skin	0	Ecchymosis	Yes
AP018	Skin	1	Injection site tenderness	Possible
AP025	Skin	0	Ecchymosis at injection site	Yes
AP026	Skin	0	Erythema at injection site	Yes
AP034	Skin	0	Pain at injection site	Yes



**Table 3**  
**ELISPOT Analysis of Anti-PSA T Cell Immune Responses**

Patient Number	Dose/ Vehicle	Response	Frequency	
			Pre-Immunization	Post- Immunization
AP-002	10 <sup>6</sup> -aqueous	-	1/985,000	1/258,571
AP-004	10 <sup>6</sup> -aqueous	-	0	1/311,111
AP-007	10 <sup>6</sup> -aqueous	+	1/46,901	1/14,804
AP-003	10 <sup>6</sup> -matrix	+	1/90,000	1/8075
AP-005	10 <sup>6</sup> -matrix	+	1/1.2x10 <sup>6</sup>	1/152,353
AP-006	10 <sup>6</sup> -matrix	+	1/93,103	1/15,762
AP-008	10 <sup>7</sup> -aqueous	-	1/100,000	1/97,419
AP-010	10 <sup>7</sup> -aqueous	-	0	0
AP-013	10 <sup>7</sup> -aqueous	+	0	1/60,000
AP-009	10 <sup>7</sup> -matrix	+	1/52,535	1/30,566
AP-012	10 <sup>7</sup> -matrix	-	1/13,488	1/254,286
AP-014	10 <sup>7</sup> -matrix	+	1/562,500	1/120,000
AP-015	10 <sup>8</sup> -aqueous	+	1/1.4x10 <sup>6</sup>	1/780
AP-016	10 <sup>8</sup> -aqueous	+	0	1/130,000
AP-017	10 <sup>8</sup> -aqueous	+	0	1/124,375
AP-025	10 <sup>8</sup> -aqueous	-	0	0
AP-026	10 <sup>8</sup> -aqueous	-	0	1/560,000
AP-027	10 <sup>8</sup> -aqueous	+	0	1/26,364
AP-029	10 <sup>8</sup> -aqueous	+	0	1/333
AP-032	10 <sup>8</sup> -aqueous	+	1/250,000	1/3793
AP-018	10 <sup>8</sup> -matrix	-	0	0
AP-019	10 <sup>8</sup> -matrix	+	1/14,235	1/336
AP-020	10 <sup>8</sup> -matrix	+	1/8824	1/1802
AP-021	10 <sup>8</sup> -matrix	+	1/689	1/431
AP-022	10 <sup>8</sup> -matrix	+	0	1/180,000
AP-023	10 <sup>8</sup> -matrix	-	1/320,000	0
AP-030	10 <sup>8</sup> -matrix	+	1/666.667	1/32,000
AP-034	10 <sup>8</sup> -matrix	+	1/80,000	1/15,152
AP-035	10 <sup>8</sup> -matrix	+	1/5295	1/2459
AP-036	10 <sup>8</sup> -matrix	+	1/62,000	1/9487
AP-037	10 <sup>8</sup> matrix	-	1/2.1x10 <sup>6</sup>	1/965,000

**Table 4**  
**PSA Doubling Time (DT) in Phase I Patients**

Vaccine Dose & Vehicle	Percent Increased PSA DT
10 <sup>6</sup> aqueous	33%
10 <sup>6</sup> matrix	33%
10 <sup>7</sup> aqueous	67%
10 <sup>7</sup> matrix	67%
10 <sup>8</sup> aqueous	25%
10 <sup>8</sup> matrix	62%
<b>Total</b>	<b>54%</b>

**Table 5**  
**Ad/PSA Phase I Trial**  
**Predicted and Actual Patient Survival**

Vaccine Dose	Vehicle	Percent with Longer Than Predicted Survival
$10^6$	Aqueous	33%
$10^6$	Matrix	67%
$10^7$	Aqueous	100%
$10^7$	Matrix	33%
$10^8$	Aqueous	56%
$10^8$	Matrix	50%
<b>Overall</b>		<b>57%</b>

June 21, 2006

David M. Lubaroff, Ph.D.  
Professor and Director of Urology Research  
Department of Urology  
University of Iowa Health Care  
375 Newton Road, 3210 MERF  
Iowa City, IA 52242

RE: Protocol #0605-780 entitled: *Phase II Study of Adenovirus/PSA Vaccine in Men with Recurrent Prostate Cancer after Local Therapy*

Dear Dr. Lubaroff:

I am writing to notify you of the outcome of the initial review of your submission by members of the National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC).

In accordance with Appendix M of the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*, members of the RAC carried out an initial review of your submission to assess whether it raised any significant issues that warrant further review and discussion by the RAC in a public session. They were provided a copy of the entire protocol submission as well as a brief summary of the submission's main features. After review of this material and other relevant information, it was determined that your submission does not require an in-depth review and public RAC discussion.

As you know, during the initial review process, RAC members may request additional information or clarification about the submission. They also may have specific comments or suggestions about the protocol design, informed consent document, or other matters. These questions and comments were conveyed to you and you were provided an opportunity to address them. Since these comments represent the considered perspectives of individual members and do not constitute a consensus of the RAC, you are encouraged, but not required, to consider them further. This correspondence becomes part of the public record of the protocol submission and is available upon request to the investigator(s), sponsor (if applicable), Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB) as well as members of the public. Requests should be directed to the NIH Office of Biotechnology Activities (OBA) via facsimiles (301-496-9839). The NIH OBA protocol number should be included in any request.

The Principal Investigator and the institution are responsible for ensuring that no research participants are enrolled in the protocol until IBC approval, IRB approval and all applicable regulatory authorizations have been obtained. Please be mindful that even though the protocol has completed the RAC review process and was not selected for in-depth review and public RAC discussion, it may still raise issues that

warrant careful consideration by the IBC and IRB. The RAC review process is not a substitute for the important institutional review of the protocol that must be carried out by the IBC and IRB.

As you proceed with the initiation of your protocol, the current reporting requirements set forth at Appendix M-I-C-1 of the *NIH Guidelines* require the Principal Investigator to submit additional documentation as specified to this office no later than 20 working days after enrollment of the first research participant. These requirements are as follows:

- a copy of the informed consent document approved by the Institutional Review Board (IRB);
- a copy of the protocol approved by the Institutional Biosafety Committee (IBC) and IRB;
- a copy of the final IBC approval from the clinical trial site;
- a copy of the final IRB approval;
- any modifications to the protocol as required by FDA;
- applicable NIH grant number(s);
- the FDA Investigational New Drug Application (IND) number; and
- the date of the initiation of the trial.

A copy of this and other sections of the *NIH Guidelines* that outline reporting requirements are enclosed.

The Internet site <<http://www4.od.nih.gov/oba/>> of the NIH OBA includes a copy of the complete *NIH Guidelines*, minutes of RAC meetings, and information about gene transfer research protocols registered with our office. Contact information for our office is as follows:

Office of Biotechnology Activities (OBA)  
National Institutes of Health  
6705 Rockledge Drive, Suite 750, MSC  
Bethesda, Maryland 20892-7985  
(All non-USPS mail should use zip code 20817)  
Phone: 301-496-9838; Fax: 301-496-9839

Please let us know if you have any questions about the review of your submission or the requirements of the *NIH Guidelines*.

Sincerely,

/s/

Thomas Y. Shih, M.D., Ph.D.  
Biotechnology Program Advisor

Attachments

cc. Louise V. Kirchhoff, M.D., IBC Chair, University of Iowa  
J. Andrew Bertolatus, M.D., IRB Chair, University of Iowa  
RAC Chair and Members  
Amy P. Patterson, M.D., Director, OBA  
Stephanie L. Simek, Ph.D., Deputy Director, Division of Cellular and Gene  
Therapies, Office of Cellular, Tissue and Gene Therapies, CBER, FDA, DHHS  
Kristina C. Borrer, Ph.D., Director, Division of Compliance Oversight, Office for Human  
Research Protections, OS, DHHS

## **OUTCOME OF THE INITIAL REVIEW BY RAC MEMBERS**

**Human Gene Transfer Protocol:** #0605-780

**Principal Investigator(s):** David M. Lubaroff, Ph.D., University of Iowa Health Care, Iowa City, IA 52242

**Submitter:** Same

**Title:** Phase II Study of Adenovirus/PSA Vaccine in Men with Recurrent Prostate Cancer after Local Therapy

**In-depth Review and Public RAC Discussion Not Required:** Nemerow, Albelda, Dewhurst, Heslop, Shapiro, Somia, Kwan, Wara, Rosenberg

**In-depth Review and Public RAC Discussion Required:** None

**Abstained:** None

**Recused:** None

**Are there comments by RAC members?** Yes

**Reporting Requirements  
of the  
NIH Guidelines for Research Involving Recombinant DNA Molecules  
(Appendix M-I-C)**

### **Appendix M-I-C-1. Initiation of the Clinical Investigation**

No later than 20 working days after enrollment (see definition of enrollment in Section I-E-7) of the first research participant in a human gene transfer experiment, the Principal Investigator(s) shall submit the following documentation to NIH OBA: (1) a copy of the informed consent document approved by the Institutional Review Board (IRB); (2) a copy of the protocol approved by the Institutional Biosafety Committee (IBC) and IRB; (3) a copy of the final IBC approval from the clinical trial site; (4) a copy of the final IRB approval; (5) a brief written report that includes the following information: (a) how the investigator(s) responded to each of the RAC's recommendations on the protocol (if applicable); and (b) any modifications to the protocol as required by FDA; (6) applicable NIH grant number(s); (7) the FDA Investigational New Drug Application (IND) number; and (8) the date of the initiation of the trial. The purpose of requesting the FDA IND number is for facilitating interagency collaboration in the Federal oversight of human gene transfer research.

### **Appendix M-I-C-2. Additional Clinical Trial Sites**

No research participant shall be enrolled (see definition of enrollment in Section I-E-7) at a clinical trial site until the following documentation has been submitted to NIH OBA: (1) Institutional Biosafety Committee approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

### **Appendix M-I-C-3. Annual Reports**

Within 60 days after the one-year anniversary of the date on which the investigational new drug (IND) application went into effect, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information set forth in (a), (b), and (c). When multiple studies are conducted under the single IND, the Principal Investigator (or delegate) may choose to submit a single annual report covering all studies, provided that each study is identified by its OBA protocol number.

(a) Clinical Trial Information. A brief summary of the status of each trial in progress and each trial completed during the previous year. The summary is required to include the following information for each trial: (1) the title and purpose of the trial; (2) clinical site; (3) the Principal Investigator; (4) clinical protocol identifiers, including the NIH OBA protocol number, NIH grant number(s) (if applicable), and the FDA IND application number; (5) participant population (such as disease indication and general age group, e.g., adult or pediatric); (6) the total number of participants planned for inclusion in the trial; the number entered into the trial to date; the number whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons; (7) the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed, and (8) if the trial has been completed, a brief description of any study results.

(b) Progress Report and Data Analysis. Information obtained during the previous year's clinical and non-clinical investigations, including: (1) a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system; (2) a summary of all serious adverse events submitted during the past year; (3) a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications; (4) if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death; and (5) a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

(c) A copy of the updated clinical protocol including a technical and non-technical abstract.

### **Appendix M-I-C-4. Safety Reporting**

Principal Investigators must submit, in accordance with this section, Appendix M-I-C-4-a and Appendix M-I-C-4-b, a written report on: (1) any serious adverse event that is both unexpected and associated with the use of the gene transfer product (i.e., there is reasonable possibility that the event may have been caused by the use of the product; investigators should not await definitive proof of association before reporting such events); and (2) any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity, teratogenicity, or carcinogenicity. The report must be clearly labeled as a "Safety Report" and must be submitted to the NIH Office of Biotechnology Activities (NIH OBA) and to the local Institutional Biosafety Committee within the timeframes set forth in Appendix M-I-C-4-b.

Principal Investigators should adhere to any other serious adverse event reporting requirements in accordance with federal regulations, state laws, and local institutional policies and procedures, as applicable.

Principal Investigators may delegate to another party, such as a corporate sponsor, the reporting functions set forth in Appendix

M, with written notification to the NIH OBA of the delegation and of the name(s), address, telephone and fax numbers of the contact(s). The Principal Investigator is responsible for ensuring that the reporting requirements are fulfilled and will be held accountable for any reporting lapses.

The three alternative mechanisms for reporting serious adverse events to the NIH OBA are: by e-mail to oba@od.nih.gov; by fax to 301-496-9839; or by mail to the Office of Biotechnology Activities, National Institutes of Health, MSC 7985, 6705 Rockledge Drive, Suite 750, Bethesda, Maryland 20892-7985.

#### **Appendix M-I-C-4-a. Safety Reporting: Content and Format**

The serious adverse event report must include, but need not be limited to: (1) the date of the event; (2) designation of the report as an initial report or a follow-up report, identification of all safety reports previously filed for the clinical protocol concerning a similar adverse event, and an analysis of the significance of the adverse event in light of previous similar reports; (3) clinical site; (4) the Principal Investigator; (5) NIH Protocol number; (6) FDA's Investigational New Drug (IND) Application number; (7) vector type, e.g., adenovirus; (8) vector subtype, e.g., type 5, relevant deletions; (9) gene delivery method, e.g., *in vivo*, *ex vivo* transduction; (10) route of administration, e.g., intratumoral, intravenous; (11) dosing schedule; (12) a complete description of the event; (13) relevant clinical observations; (14) relevant clinical history; (15) relevant tests that were or are planned to be conducted; (16) date of any treatment of the event; and (17) the suspected cause of the event. These items may be reported by using the recommended Adverse Event Reporting Template available on NIH OBA's web site at: <http://www4.od.nih.gov/oba/rac/documents1.htm>, the FDA MedWatch forms, or other means provided that all of the above elements are specifically included.

Reports from laboratory animal studies as delineated in Appendix M-I-C-4 must be submitted in a narrative format.

#### **Appendix M-I-C-4-b. Safety Reporting: Time frames for Expedited Reports**

Any serious adverse event that is fatal or life-threatening, that is unexpected, and associated with the use of the gene transfer product must be reported to the NIH OBA as soon as possible, but not later than 7 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

Serious adverse events that are unexpected and associated with the use of the gene transfer product, but are not fatal or life-threatening, must be reported to the NIH OBA as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

Changes in this schedule are permitted only where, under the FDA IND regulations [21 CFR 312(c)(3)], changes in this reporting schedule have been approved by the FDA and are reflected in the protocol.

If, after further evaluation, an adverse event initially considered not to be associated with the use of the gene transfer product is subsequently determined to be associated, then the event must be reported to the NIH OBA within 15 days of the determination.

Relevant additional clinical and laboratory data may become available following the initial serious adverse event report. Any follow-up information relevant to a serious adverse event must be reported within 15 calendar days of the sponsor's receipt of the information. If a serious adverse event occurs after the end of a clinical trial and is determined to be associated with the use of the gene transfer product, that event shall be reported to the NIH OBA within 15 calendar days of the determination.

Any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity, teratogenicity, or carcinogenicity must be reported as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

#### **Appendix M-I-C-5. Confidentiality**

Data submitted in accordance with Appendix M-I-C that are claimed to be confidential commercial or trade secret information must be clearly labeled as such. Prior to making its determination about the confidentiality of data labeled confidential commercial or trade secret, the NIH will contact the Principal Investigator or delegate to ascertain the basis for the claim and subsequently will notify the Principal Investigator or delegate of its final determination regarding the claim.

If NIH determines that the data so labeled are confidential commercial or trade secret and that their public disclosure would promote an understanding of key scientific or safety issues, the NIH will seek agreement from the appropriate party to release such data. Public discussion of scientific and safety issues raised by data submitted in accordance with Appendix M-I-C is vital to informing both investigators and human subjects about the safety of gene transfer research.

To protect the privacy of participants in gene transfer research, any serious adverse event or annual reports submitted to NIH OBA must not contain any information that would identify the human research participants.

June 21, 2006

David M. Lubaroff, Ph.D.  
Professor and Director of Urology Research  
Department of Urology  
University of Iowa Health Care  
375 Newton Road, 3210 MERF  
Iowa City, IA 52242

RE: Protocol #0605-781 entitled: *Phase II Study of Adenovirus/PSA Vaccine in Men with Hormone – Refractory Prostate Cancer*

Dear Dr. Lubaroff:

I am writing to notify you of the outcome of the initial review of your submission by members of the National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC).

In accordance with Appendix M of the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*, members of the RAC carried out an initial review of your submission to assess whether it raised any significant issues that warrant further review and discussion by the RAC in a public session. They were provided a copy of the entire protocol submission as well as a brief summary of the submission's main features. After review of this material and other relevant information, it was determined that your submission does not require an in-depth review and public RAC discussion.

As you know, during the initial review process, RAC members may request additional information or clarification about the submission. They also may have specific comments or suggestions about the protocol design, informed consent document, or other matters. These questions and comments were conveyed to you and you were provided an opportunity to address them. Since these comments represent the considered perspectives of individual members and do not constitute a consensus of the RAC, you are encouraged, but not required, to consider them further. This correspondence becomes part of the public record of the protocol submission and is available upon request to the investigator(s), sponsor (if applicable), Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB) as well as members of the public. Requests should be directed to the NIH Office of Biotechnology Activities (OBA) via facsimiles (301-496-9839). The NIH OBA protocol number should be included in any request.

The Principal Investigator and the institution are responsible for ensuring that no research participants are enrolled in the protocol until IBC approval, IRB approval and all applicable regulatory authorizations have been obtained. Please be mindful that even though the protocol has completed the RAC review process and was not selected for in-depth review and public RAC discussion, it may still raise issues that warrant careful consideration by the IBC and IRB. The RAC review process is not a substitute for the important institutional review of the protocol that must be carried out by the IBC and IRB.



As you proceed with the initiation of your protocol, the current reporting requirements set forth at Appendix M-I-C-1 of the *NIH Guidelines* require the Principal Investigator to submit additional documentation as specified to this office no later than 20 working days after enrollment of the first research participant. These requirements are as follows:

- a copy of the informed consent document approved by the Institutional Review Board (IRB);
- a copy of the protocol approved by the Institutional Biosafety Committee (IBC) and IRB;
- a copy of the final IBC approval from the clinical trial site;
- a copy of the final IRB approval;
- any modifications to the protocol as required by FDA;
- applicable NIH grant number(s);
- the FDA Investigational New Drug Application (IND) number; and
- the date of the initiation of the trial.

A copy of this and other sections of the *NIH Guidelines* that outline reporting requirements are enclosed.

The Internet site <<http://www4.od.nih.gov/oba/>> of the NIH OBA includes a copy of the complete *NIH Guidelines*, minutes of RAC meetings, and information about gene transfer research protocols registered with our office. Contact information for our office is as follows:

Office of Biotechnology Activities (OBA)  
National Institutes of Health  
6705 Rockledge Drive, Suite 750, MSC  
Bethesda, Maryland 20892-7985  
(All non-USPS mail should use zip code 20817)  
Phone: 301-496-9838; Fax: 301-496-9839

Please let us know if you have any questions about the review of your submission or the requirements of the *NIH Guidelines*.

Sincerely,

/s/

Thomas Y. Shih, M.D., Ph.D.  
Biotechnology Program Advisor

Attachments

cc. Louise V. Kirchhoff, M.D., IBC Chair, University of Iowa  
J. Andrew Bertolatus, M.D., IRB Chair, University of Iowa  
RAC Chair and Members  
Amy P. Patterson, M.D., Director, OBA  
Stephanie L. Simek, Ph.D., Deputy Director, Division of Cellular and Gene  
Therapies, Office of Cellular, Tissue and Gene Therapies, CBER, FDA, DHHS  
Kristina C. Borrer, Ph.D., Director, Division of Compliance Oversight, Office for Human  
Research Protections, OS, DHHS

## **OUTCOME OF THE INITIAL REVIEW BY RAC MEMBERS**

**Human Gene Transfer Protocol:** #0605-781

**Principal Investigator(s):** David M. Lubaroff, Ph.D., University of Iowa Health Care, Iowa City, IA 52242

**Submitter:** Same

**Title:** Phase II Study of Adenovirus/PSA Vaccine in Men with Hormone – Refractory Prostate Cancer

**In-depth Review and Public RAC Discussion Not Required:** Albelda, Nemerow, Dewhurst, Heslop, Shapiro, Muzyczka, Somia, Kwan, Wara, Rosenberg

**In-depth Review and Public RAC Discussion Required:** None

**Abstained:** None

**Recused:** None

**Are there comments by RAC members?** No

**Reporting Requirements  
of the  
NIH Guidelines for Research Involving Recombinant DNA Molecules  
(Appendix M-I-C)**

**Appendix M-I-C-1. Initiation of the Clinical Investigation**

No later than 20 working days after enrollment (see definition of enrollment in Section I-E-7) of the first research participant in a human gene transfer experiment, the Principal Investigator(s) shall submit the following documentation to NIH OBA: (1) a copy of the informed consent document approved by the Institutional Review Board (IRB); (2) a copy of the protocol approved by the Institutional Biosafety Committee (IBC) and IRB; (3) a copy of the final IBC approval from the clinical trial site; (4) a copy of the final IRB approval; (5) a brief written report that includes the following information: (a) how the investigator(s) responded to each of the RAC's recommendations on the protocol (if applicable); and (b) any modifications to the protocol as required by FDA; (6) applicable NIH grant number(s); (7) the FDA Investigational New Drug Application (IND) number; and (8) the date of the initiation of the trial. The purpose of requesting the FDA IND number is for facilitating interagency collaboration in the Federal oversight of human gene transfer research.

**Appendix M-I-C-2. Additional Clinical Trial Sites**

No research participant shall be enrolled (see definition of enrollment in Section I-E-7) at a clinical trial site until the following documentation has been submitted to NIH OBA: (1) Institutional Biosafety Committee approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

**Appendix M-I-C-3. Annual Reports**

Within 60 days after the one-year anniversary of the date on which the investigational new drug (IND) application went into effect, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information set forth in (a), (b), and (c). When multiple studies are conducted under the single IND, the Principal Investigator (or delegate) may choose to submit a single annual report covering all studies, provided that each study is identified by its OBA protocol number.

(a) Clinical Trial Information. A brief summary of the status of each trial in progress and each trial completed during the previous year. The summary is required to include the following information for each trial: (1) the title and purpose of the trial; (2) clinical site; (3) the Principal Investigator; (4) clinical protocol identifiers, including the NIH OBA protocol number, NIH grant number(s) (if applicable), and the FDA IND application number; (5) participant population (such as disease indication and general age group, e.g., adult or pediatric); (6) the total number of participants planned for inclusion in the trial; the number entered into the trial to date; the number whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons; (7) the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed, and (8) if the trial has been completed, a brief description of any study results.

(b) Progress Report and Data Analysis. Information obtained during the previous year's clinical and non-clinical investigations, including: (1) a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system; (2) a summary of all serious adverse events submitted during the past year; (3) a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications; (4) if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death; and (5) a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

(c) A copy of the updated clinical protocol including a technical and non-technical abstract.

**Appendix M-I-C-4. Safety Reporting**

Principal Investigators must submit, in accordance with this section, Appendix M-I-C-4-a and Appendix M-I-C-4-b, a written report on: (1) any serious adverse event that is both unexpected and associated with the use of the gene transfer product (i.e., there is reasonable possibility that the event may have been caused by the use of the product; investigators should not await definitive proof of association before reporting such events); and (2) any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity, teratogenicity, or carcinogenicity. The report must be clearly labeled as a "Safety Report" and must be submitted to the NIH Office of Biotechnology Activities (NIH OBA) and to the local Institutional Biosafety Committee within the timeframes set forth in Appendix M-I-C-4-b.

Principal Investigators should adhere to any other serious adverse event reporting requirements in accordance with federal regulations, state laws, and local institutional policies and procedures, as applicable.

Principal Investigators may delegate to another party, such as a corporate sponsor, the reporting functions set forth in Appendix

M, with written notification to the NIH OBA of the delegation and of the name(s), address, telephone and fax numbers of the contact(s). The Principal Investigator is responsible for ensuring that the reporting requirements are fulfilled and will be held accountable for any reporting lapses.

The three alternative mechanisms for reporting serious adverse events to the NIH OBA are: by e-mail to oba@od.nih.gov; by fax to 301-496-9839; or by mail to the Office of Biotechnology Activities, National Institutes of Health, MSC 7985, 6705 Rockledge Drive, Suite 750, Bethesda, Maryland 20892-7985.

#### **Appendix M-I-C-4-a. Safety Reporting: Content and Format**

The serious adverse event report must include, but need not be limited to: (1) the date of the event; (2) designation of the report as an initial report or a follow-up report, identification of all safety reports previously filed for the clinical protocol concerning a similar adverse event, and an analysis of the significance of the adverse event in light of previous similar reports; (3) clinical site; (4) the Principal Investigator; (5) NIH Protocol number; (6) FDA's Investigational New Drug (IND) Application number; (7) vector type, e.g., adenovirus; (8) vector subtype, e.g., type 5, relevant deletions; (9) gene delivery method, e.g., *in vivo*, *ex vivo* transduction; (10) route of administration, e.g., intratumoral, intravenous; (11) dosing schedule; (12) a complete description of the event; (13) relevant clinical observations; (14) relevant clinical history; (15) relevant tests that were or are planned to be conducted; (16) date of any treatment of the event; and (17) the suspected cause of the event. These items may be reported by using the recommended Adverse Event Reporting Template available on NIH OBA's web site at: <http://www4.od.nih.gov/oba/rac/documents1.htm>, the FDA MedWatch forms, or other means provided that all of the above elements are specifically included.

Reports from laboratory animal studies as delineated in Appendix M-I-C-4 must be submitted in a narrative format.

#### **Appendix M-I-C-4-b. Safety Reporting: Time frames for Expedited Reports**

Any serious adverse event that is fatal or life-threatening, that is unexpected, and associated with the use of the gene transfer product must be reported to the NIH OBA as soon as possible, but not later than 7 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

Serious adverse events that are unexpected and associated with the use of the gene transfer product, but are not fatal or life-threatening, must be reported to the NIH OBA as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

Changes in this schedule are permitted only where, under the FDA IND regulations [21 CFR 312(c)(3)], changes in this reporting schedule have been approved by the FDA and are reflected in the protocol.

If, after further evaluation, an adverse event initially considered not to be associated with the use of the gene transfer product is subsequently determined to be associated, then the event must be reported to the NIH OBA within 15 days of the determination.

Relevant additional clinical and laboratory data may become available following the initial serious adverse event report. Any follow-up information relevant to a serious adverse event must be reported within 15 calendar days of the sponsor's receipt of the information. If a serious adverse event occurs after the end of a clinical trial and is determined to be associated with the use of the gene transfer product, that event shall be reported to the NIH OBA within 15 calendar days of the determination.

Any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity, teratogenicity, or carcinogenicity must be reported as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

#### **Appendix M-I-C-5. Confidentiality**

Data submitted in accordance with Appendix M-I-C that are claimed to be confidential commercial or trade secret information must be clearly labeled as such. Prior to making its determination about the confidentiality of data labeled confidential commercial or trade secret, the NIH will contact the Principal Investigator or delegate to ascertain the basis for the claim and subsequently will notify the Principal Investigator or delegate of its final determination regarding the claim.

If NIH determines that the data so labeled are confidential commercial or trade secret and that their public disclosure would promote an understanding of key scientific or safety issues, the NIH will seek agreement from the appropriate party to release such data. Public discussion of scientific and safety issues raised by data submitted in accordance with Appendix M-I-C is vital to informing both investigators and human subjects about the safety of gene transfer research.

To protect the privacy of participants in gene transfer research, any serious adverse event or annual reports submitted to NIH OBA must not contain any information that would identify the human research participants.